

A New Saponin from the Seeds of *Heterophragma quadriloculare*

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*H. quadriloculare*¹ (Bignoniaceae) occurs in M.P., Deccan and Southern region of the country. The roots of the plant are reported to be useful in snake-bite.

The air-dried powdered and defatted seeds (2 kg) of *H. quadriloculare* were extracted with 95% ethanol for several hours. The ethanolic extract was concentrated under reduced pressure and to it was added large amount of acetone to get a precipitate which was separated by filtration. The process of dissolution in alcohol and precipitation by acetone was repeated several times to get a light brown amorphous powder which gave all the tests for saponins². On tlc examination, it showed single spot which was purified by column chromatography over silica-gel G and crystallised as brown needles from acetone-ethanol (1 : 1), $C_{40}H_{88}O_{10}$, M^+ 698, m.p. 141–42°; its acetyl derivative, $C_{42}H_{86}O_{11}$, m.p. 139–40°, acetyl (5.4%), M^+ 740; its $\nu_{\max}$ (KBr) 3 180, 2 905, 1 640, 1 450, 1 380, 1 130, 1 065, 1 025, 998, 955, 840 and 805 cm^{-1} and ^1H nmr spectra of acetyl derivative of the glucoside δ ($CDCl_3$; TMS) at 0.75 (3H, s, CH_3 -18), 0.67 (6H, dd, J 6,6,2, secondary CH_3 at 25, 26), 0.72 (3H, d, J 5.2 Hz, CH_3 -20), 0.78 (3H, d, J 6 Hz, CH_3 of rhamnose), 0.82 (3H, m- CH_3 -27), 1.34–1.92 (29H, complex pattern for polymethylene CH_2 and CH), 2.12 (3H, s, OAc-3), 2.02, 2.04, 2.05, 2.06, 2.08, 2.10 (6-OAc of glucose and rhamnose), 3.52–4.25 (4H, m, protons of glucose), 4.35 (1H, d, J 5.6 Hz, 1'-anomeric proton), 4.46 (1H, t, H-3), 4.56–4.68 (4H, m, protons of rhamnose), 5.48 (1H, d, J 2 Hz, anomeric proton) and 5.50 (1H, dd, J 4.5, 8.5 Hz, H-6).

On hydrolysis with 7% H_2SO_4 , it afforded a

genin, m.p. 156–57°, $[\alpha]_D^{25}$ –31.6, $C_{28}H_{48}O$, M^+ 400, crystallised from ethanol-acetone (1 : 1) as colourless needles. It formed an acetate, m.p. 158–59°, $[\alpha]_D^{25}$ = 9.1 ($CHCl_3$), $C_{28}H_{48}O_2$, M^+ 442; $\nu_{\max}$ (KBr) 3 400, 2 905, 1 635, 1 450, 1 390, 1 130, 1 065, 1 025, 998, 955, 840 and 810 cm^{-1} ; m/z 400, 385, 382, 315 and 230. It was identified as 24-methylcholest-5-ene-3 β -ol (m.p., m.m.p., co-pc and tlc) with authentic samples. The hydrolysate was neutralised with $BaCO_3$ and $BaSO_4$ and was filtered off. The filtrate was concentrated to a brown viscous mass and was found to contain two sugars, D-glucose and L-rhamnose³ (co-pc and co-tlc) with authentic samples. Permethylatation⁴ of the saponin followed by acid hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-rhamnose, thereby confirming that C_1 of the rhamnose was involved in the glycosylation. Periodate oxidation⁵ of the saponin suggested that both the sugars are present in the pyranose form.

Partial hydrolysis of the saponin with Killiani mixture liberated D-glucose followed by L-rhamnose, suggesting that D-glucose was the terminal sugar and L-rhamnose was attached to genin. Enzyme emulsin⁶ hydrolysis confirmed the presence of β -linkage between L-rhamnose and the genin and hydrolysis with the enzyme tokadiastase confirmed α -linkage between D-glucose and the L-rhamnose. Therefore, sapogenin was linked at C_1 of the L-rhamnose via β -linkage and C_4 of the L-rhamnose was attached to the C_1 of the D-glucose via α -linkage. Thus saponin was identified as 24-methylcholest-5-en-3-*O*- α -D-glucoside (1 $\rightarrow$ 4)-*O*- β -L-rhamnoside.

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