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STIGMASTA-5,24(28)-DIENE-3β-*O*-α-L-RHAMNOSIDE FROM *CLEOME VISCOSA*

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INTRODUCTION

Cleome viscosa [1, 2] is a member of a genus of plants used especially in the indigenous system of medicine. Previous work on this plant was reported by Srivastava *et al.* [3, 4]. In the present paper I have reported the isolation of a new saponin identified as stigmasta-5,24(28)-diene-3β-*O*-α-L-rhamnoside (**1**) on the basis of chemical and spectral evidence.

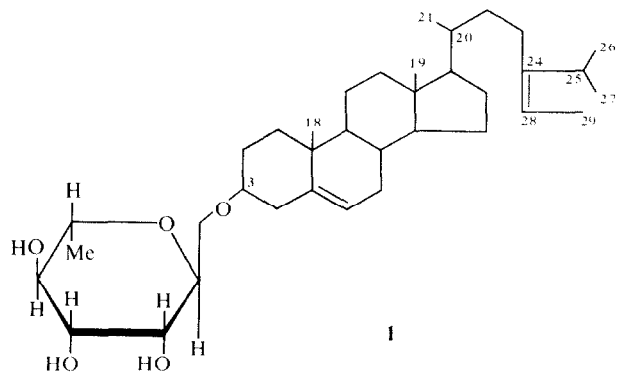
RESULTS AND DISCUSSION

The saponin, mp 60–62, gave a copious lather when shaken with H_2O, haemolysed red blood cells and was toxic to fish. On acid hydrolysis, the saponin afforded a genin, mp 122–124°, $C_{29}H_{48}O$ (M^+ at *m/e* 412), $[\alpha]_D^{25}$ − 38° (in $CHCl_3$) and a sugar L-rhamnose (co-PPC and osazone). The IR spectrum (KBr, cm^{-1}) showed absorptions at 3420 (OH group); 2952, 1650, 1575 and 800 (strong) ($\Delta^{24(28)}$ ethylidene sterol) [5, 6]; 1475, 1390 and 955 (*iso*-propyl group). The ^1H NMR spectrum exhibited signals ($CDCl_3$, TMS) at δ 0.81 (*s*, Me-19); 0.99 (fused *d*, Me-21, 26 and 27); 1.02 (*s*, Me-18); 1.55 (*d*, Me-29); 2.81 (*septet*, H-25); and 5.02 (*q*, H-28). The MS of the genin showed mass fragments at *m/e* 412 (M^+, parent peak); 397 (M^+ − Me); 394 (M^+ − H_2O); 369 (M^+ − C_3H_7); 314 (M^+ − C_7H_{14}); 299 [M^+ − (C_7H_{14} + Me)]; 287 [M^+ − (Me + C_8H_{15} − H)]; 273 (M^+ − side chain) i.e. (M^+ − $C_{10}H_{19}$): 257 [larger ion, M^+ − ($C_{11}H_{21}$ + 2H)] and 253 [smaller ion, M^+ − (side chain + 2H + H_2O)]. The genin formed a monoacetate, mp 118–119° [$C_{31}H_{50}O_2$ (M^+ at *m/e* 454), $[\alpha]_D^{25}$ − 45° (in $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 1720; ^1H NMR, δ at 2.09 (−COCH$_3$)], a benzoate, mp 118–20° [$C_{36}H_{52}O_2$ (M^+ at *m/e* 516), $[\alpha]_D^{25}$ − 16° (in $CHCl_3$)] and a digitonide, mp 233–235° showing the presence of a OH function in the molecule. The genin on Oppenauer oxidation gave stigmasta-4,24(28)-dien-3-one (α,β unsaturated ketone) mp 93–94° indicating the presence of Δ^5-3β-OH grouping [5]. Ozonolysis of the genin gave acetaldehyde which located the second double bond probably at the $\Delta^{24(28)}$ position [6]. On partial hydrogenation the genin afforded two products identified as a mixture of sitosterol [7] and clionasterol [8], (mp, mmp and co-TLC) mp 133°, $[\alpha]_D^{25}$ − 30° (in $CHCl_3$). From the above discussion it is clear that the genin was stigmasta-5,24(28)-dien-3β-ol which was further confirmed by co-chromatography with an authentic sample [9].

Periodate oxidation showed the consumption of 2.01 mol of periodate with the liberation of 1.01 mol of HCO_2H per 1 mol of the saponin suggesting the presence of one unit of rhamnose in pyranose form. Enzymatic hydrolysis proved the α-linkage between the genin and L-rhamnose. Thus the saponin was stigmasta-5,24(28)-diene-3β-*O*-α-L-rhamnopyranoside (**1**).

EXPERIMENTAL

Isolation and purification. Air-dried and powdered whole plant of *C. viscosa* (2 kg) procured from the United Chemicals and Allied Products, Calcutta (India) was extracted with EtOH under reflux for 160 hr. The ethanolic extract (2.5 l.) was concd (100 ml) under red. pres. It was segregated into H_2O soluble and insoluble material by pouring into H_2O (500 ml.). The H_2O soluble portion was concd and subjected to liquid liquid extraction with C_6H_6. The C_6H_6 extract was chromatographed on a neutral Al_2O_3



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column ($CHCl_3$–C_6H_6; 5:5) to afford a white amorphous residue (1.2 g) which was crystallized from $CHCl_3$–MeOH (2:3) into white needles, mp 60–62°, homogeneous on TLC [R_f 0.52 (in $CHCl_3$–MeOH; 9.5:0.5) and 0.34 in BAW (4:1:5)]; λ_{max}^{EtOH} 205 nm. (Found: C, 75.25; H, 10.60: $C_{35}H_{58}O_5$ requires; C, 75.26; H, 10.39%).

Acid hydrolysis of saponin. The saponin (1.0 g) was refluxed with 7% EtOH–H_2SO_4 (100 ml) for 5 hr, concd, diluted with H_2O and cooled to give genin as a white amorphous solid (800 mg) which was crystallized as colourless needles with $CHCl_3$–MeOH, mp 122–124°. The aq. hydrolysate after neutralization ($BaCO_3$) was concd to a syrup, which was identified as L-rhamnose by the usual methods. Quantitative analysis [10] revealed the presence of 1 mol of rhamnose.

Study of the genin. The genin (800 mg) was crystallized as colourless needles from $CHCl_3$–MeOH (9:1), λ_{max}^{EtOH} 203 nm. (Found: C, 84.42; H, 11.66. $C_{29}H_{48}O$ requires: C, 84.46; H, 11.65%). The genin (100 mg) was acetylated with Ac_2O (2 ml) and C_5H_5N (5 ml) by the usual process and the percentage in the acetylated product was determined as in ref. [11, 12]. (Found: C, 81.90; H, 11.00; OAc, 9.45. $C_{31}H_{50}O_2$ requires: C, 81.93; H, 11.00; 1 × OAc, 9.44%). M^+ at *m/e* 454. The genin (100 mg) was benzoylated with PhCOCl (2 ml) and C_5H_5N (5 ml) in the usual manner. (Found: C, 83.70; H, 10.06; $C_{36}H_{52}O_2$ required; C, 83.72; H, 10.07%). M^+ at *m/e* 516.

Oppenauer oxidation of the genin. The genin (100 mg) in dry Me_2CO (5 ml) was mixed with $[(Me_3)_3CO]_3Al$ (120 mg) in C_6H_6 (5 ml) and refluxed for 20 hr. The reaction mixture was washed with $2NH_2SO_4$, H_2O and $NaHCO_3$, respectively. The product was crystallized as colourless plates with Me_2CO, mp 93–95°, $[\alpha]_D^{25}$ + 77° (in $CHCl_3$) which was identified as stigmasta-4,24(28)-dien-3-one (60 mg). (Found: C, 84.88; H, 11.20. $C_{29}H_{46}O$ requires: C, 84.87; H, 11.21%).

Ozonization of the genin. The genin (200 mg) in glacial HOAc (10 ml) was ozonized for 2 hr at an O_3 concn of *ca* 2%, the exit gases being led through 20 ml H_2O. The reaction mixture was passed through a Si gel column, 20 ml distillate were collected and a soln of 100 mg recrystallized *p*-NO_2-Ph-$NHNH_2$ in 10 ml 50% HOAc was gradually added. The hydrazone crystallized out immediately and was recrystallized from EtOH, mp 127–129°. It was identified as acetaldehyde-*p*-nitrophenyl-hydrazone (TLC and mmp). (Found: C, 53.62; H, 5.03; N, 23.45. $C_8H_9N_3O_2$ requires: C, 53.63; H, 5.02; N, 23.46%).

Periodate oxidation of the saponin. An EtOH soln (5%) of the saponin (20 mg) and 0.1 M $NaIO_4$ (25 ml) were mixed and allowed to stand in the dark for 48 hr. A blank was also run simultaneously. The amounts of periodate consumed and HCO_2H liberated were estimated [13] and corresponded to 2.01 and 1.01 mol respectively per 1 mol of the saponin.

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