

# STRUCTURE OF ZIZOGENIN, A NEW SAPOGENIN FROM *ZIZYPHUS MAURITANIA*

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**Key Word Index**—*Zizyphus mauritiana*; Rhamnaceae; sapogenin; zizogenin.

## INTRODUCTION

The plant *Zizyphus mauritiana* (Rhamnaceae) is known for its medicinal importance [1, 2]. The earlier work on the stem of this plant was reported by Chauhan and Srivastava [3]. In this paper we report the isolation and identification of a new sapogenin named zizogenin.

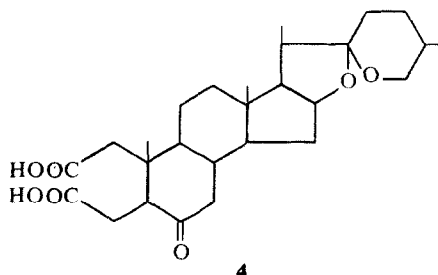
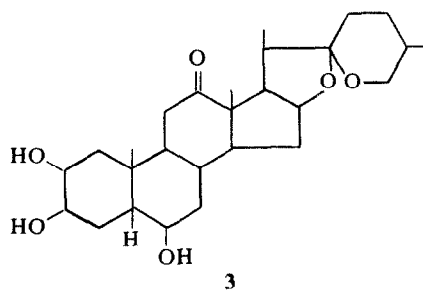
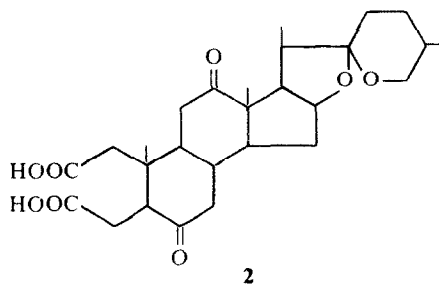
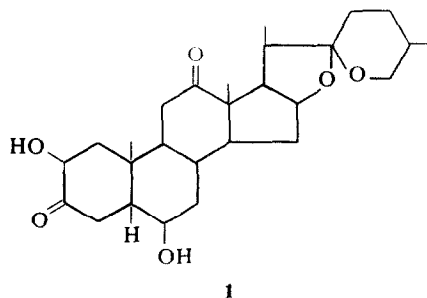
## RESULTS AND DISCUSSION

The sapogenin, mp 245–48°,  $[\alpha]_D^{25} -55^\circ$  (in  $\text{CHCl}_3$ ), molecular formula  $\text{C}_{27}\text{H}_{44}\text{O}_6$ ,  $\text{M}^+$  464, gave all the positive tests for a keto steroidal sapogenin. Its MS showed mass fragments at  $m/e$  464 (parent peak), 449 ( $\text{M} - \text{Me}$ ), 446 ( $\text{M} - \text{H}_2\text{O}$ ), 431 ( $\text{M} - \text{Me} - \text{H}_2\text{O}$ ), 189 and 187 (base peak). Its IR spectrum (in KBr) showed peaks at 850, 900, 922 and  $990\text{ cm}^{-1}$  which are characteristic of a normal sapogenin [4]. (Band 922 was stronger than the  $900\text{ cm}^{-1}$  band.) In addition to the above IR absorptions, the sapogenin showed the bands in the range  $1360\text{--}800\text{ cm}^{-1}$  which are characteristic of a sapogenin with a spiroketal side chain [5, 6]. It formed a diacetate ( $\text{Ac}_2\text{O/Py}$ ), mp  $122^\circ(\text{d})$ ,  $\text{M}^+$  548, as shown by the appearance of the acetyl absorption at  $1720\text{ cm}^{-1}$  and disappearance of the OH absorption ( $3500\text{ cm}^{-1}$ ) in the IR spectrum. Further the genin formed a 2,4-dinitrophenylhydrazone derivative ( $\nu_{\text{max}}$   $1700\text{ cm}^{-1}$ ) showing the presence of two ketonic functions in the molecule. The sapogenin gave the Zimmerman colour test [7, 8] which located one of the ketonic functions at position C-3 in the genin molecule. The sapogenin (1), on oxidation with chromic acid, afforded a compound, mp  $260^\circ(\text{d})$  which was identified as hecogenin acid (2). In hecogenic acid, a ketonic group is present at C-12 which is an inert position and accordingly the genin contained the other  $-\text{CO}$  group at this position ( $\nu_{\text{max}}$   $1040\text{--}1075\text{ cm}^{-1}$ ). The ketonic function at position C-12 was further proved by the fact that the genin molecule could not be reduced by Clemmenson reduction. On Meerwein–Ponndorf–Verley reduction (aluminium isopropoxide in isopropanol solution), the sapogenin afforded a compound, mp  $277\text{--}79^\circ$  identified as cocogenin (3) [9]. Thus the two hydroxyl groups must be present at positions C-2 and C-5 in the genin molecule. These positions were further confirmed by the fact that Wolff–Kishner reduction of the sapogenin afforded another compound, mp  $234^\circ$ , identified as chlorogenic acid (4) [9].

Thus from the above discussion, it is clear that the sapogenin, zizogenin, should be assigned structure 1.

## EXPERIMENTAL

*Isolation and purification.* Dried and powdered stem (5 kg)



of *Zizyphus mauritiana*, collected from Allahabad city, Allahabad and identified by Botanical Survey of India, Allahabad Circle, were extracted with EtOH under reflux for 20 days. The extract (4 l) was filtered off and the EtOH was distilled off to leave a solid. The solid was extracted with EtOAc to give a dark red soln which was concd to half of its volume and kept in a refrigerator for a few days, when colourless crystals deposited. The remaining filtrate was again concd to a small volume (200 ml) and kept in a refrigerator for a few more days to give a second crop of crystals. The total crystals (1.950 g) were then purified on a column of neutral  $\text{Al}_2\text{O}_3$  eluted with MeOH and crystallized from MeOH- $\text{CHCl}_3$  (9:1) into long crystals, mp 245–48°; homogeneous on TLC developed with  $\text{CHCl}_3$ -MeOH (5:5)  $R_f$  0.43 and  $\text{C}_6\text{H}_6$ -MeOH (4:6)  $R_f$  0.68.  $M^+$   $m/e$  464.  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3500, 2950, 2880, 1700, 1425, 1360, 1275, 1025, 990, 900, 922, 850, 800 and 725. (Found: C, 69.81; H, 9.48.  $\text{C}_{27}\text{H}_{44}\text{O}_6$  required: C, 69.82; H, 9.48 %).

**Acetylation of the sapogenin.** The compound (100 mg) was acetylated with 5 ml  $\text{Ac}_2\text{O}$  and 5 ml Py in the usual manner. The acetyl derivative was crystallized from  $\text{Me}_2\text{CO}$  into colourless needles, mp 122° (d). The acetyl content was determined by the method of ref. [10] as described in ref. [11]. (Found: C, 67.86; H, 8.77; acetate, 15.68.  $\text{C}_{31}\text{H}_{48}\text{O}_8$  requires: C, 67.88; H, 8.76; 2 × acetate: 15.69 %).  $M^+$   $m/e$  548.

**2,4-Dinitrophenylhydrazone derivative of the sapogenin.** The genin (200 mg) was dissolved in 30 ml of hot EtOH and to it was added a soln of 200 mg 2,4-DNP in 50 ml EtOH containing 1 ml HCl. Within a few min needles separated out. After standing at room temp. for 4 hr, the crystals were filtered and washed with cold EtOH. It was crystallized from EtOH as orange needles mp 222–225° (d).

**Zimmermann colour test of the sapogenin.** The genin (5 mg) was dissolved in 1 ml 2N KOH in absolute EtOH and 1 ml 1% *m*-dinitrobenzene in absolute EtOH was added to it. After 10 min the mixture was diluted to 10 ml with absolute EtOH when a violet colour appeared which faded after dilution.

**Oxidation of the sapogenin.** The genin (200 mg) in 15 ml HOAc was added to a soln of 200 mg chromic anhydride in 2 ml 90% HOAc. The reaction mixture was kept at room temp. for 2 hr,  $\text{H}_2\text{O}$  was added and the product was extracted with  $\text{Et}_2\text{O}$  and washed with  $\text{H}_2\text{O}$ . It was extracted with KOH and the alkaline layer was acidified with HCl and extracted with  $\text{Et}_2\text{O}$ . The solvent was removed and the residue was dissolved in 25 ml EtOH to which was added 1.4 g 2 mesh Zn. The mixture was heated under reflux on a steam bath and 1 ml conc HCl was added after 1 hr. The reaction mixture was poured into  $\text{H}_2\text{O}$  and extracted with  $\text{Et}_2\text{O}$ . The solvent was removed and the product was hydrolysed with ethanolic KOH for 1 hr. The soln was acidified and extracted with  $\text{Et}_2\text{O}$ . It was concd and kept in a freezer to crystallize out the product. It was recrystallized from  $\text{Et}_2\text{O}$ , mp and mmp identical with hecogenic

acid, 268 (d). (Found: C, 68.00; H, 8.40.  $\text{C}_{27}\text{H}_{40}\text{O}_7$  required: C, 67.94; H, 8.40 %).

**Attempted mild Clemmensen reaction with the sapogenin.** To an alcoholic soln of 100 mg of the sapogenin in 20 ml EtOH was added 2 g 2 mesh Zn. The mixture was heated under reflux and 1 ml conc HCl was added every 1 hr for 8 hr. The reaction mixture was poured into  $\text{H}_2\text{O}$  and extracted with  $\text{Et}_2\text{O}$ . After washing with  $\text{H}_2\text{O}$ , the  $\text{Et}_2\text{O}$  was concd and cooled to give crystals, mp 245–48°. No mp depression was obtained when mixed with the original sapogenin.

**Chlorogenic acid from the sapogenin.** A soln of 1 g Na in 20 ml absolute EtOH was added to 200 mg of the genin and 2 ml 85% hydrazine hydrate. The mixture was heated in a bomb-tube at 200° for 10 hr. After cooling, it was poured into  $\text{H}_2\text{O}$  and the ppt. extracted with  $\text{Et}_2\text{O}$ . The  $\text{Et}_2\text{O}$  soln was washed with  $\text{H}_2\text{O}$  and dil HCl. On cooling, a product was obtained which was crystallized from  $\text{Et}_2\text{O}$ , mp 262° (d). The product (100 mg) in 2 ml HOAc was added to a soln of 100 mg chromic anhydride in 10 ml 80% HOAc. After standing 2 hr at room temp.,  $\text{H}_2\text{O}$  was added and the product was extracted with  $\text{Et}_2\text{O}$ . The solvent was removed and the product obtained was identified as chlorogenic acid, mp 234°. (Found: C, 68.00; H, 8.50.  $\text{C}_{27}\text{H}_{40}\text{O}_7$  required: C, 67.90; H, 8.40 %).

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