## 169. *Sapogenins. Part IV. The Sapogenin of* Balanites ægyptica *Wall.*

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A new sapogenin, *nitogenin*, has been isolated from the saponin occurring in the seed kernels of *Balanites ægyptica*. It appears to belong to the group of steroid sapogenins and is very closely related to tigogenin, but is not identical with it.

The seeds kernels of *Balanites ægyptica*, a tree occurring in Northern Africa and Uganda, have long been known to contain a water-soluble constituent toxic to cold-blooded animals and have for this reason been recommended for the extermination of fresh-water snails, which are the hosts of the *Bilharzia* liver fluke. The toxic constituent has been described as a saponin (Weil, *Arch. Pharm.*, 1901, **239**, 363), but no study has been made of its chemistry.

Through the kindness of Mr. H. J. Holman, B.Sc., of the Imperial Institute we were able to examine the remainder of a batch of " dates " obtained from Entebbe, Uganda, where they are known by the native name " Lugba " (compare *Bull. Imp. Inst.*, 1935, **33**, 274). The kernels contained a considerable quantity of an amorphous saponin, which was hydrolysed to the aglucone, *nitogenin*, $C_{27}H_{44}O_3$. This compound is saturated and neutral and has one hydroxyl group, as shown by the formation of an *acetate* and a *benzoate*; the remaining oxygen atoms are inert. The compound thus appears to be a steroid sapogenin isomeric with sarsasapogenin and tigogenin. It was at first thought to be identical with the latter, as it melts only 4° lower, the benzoate has the same melting point (compare Jacobs and Fleck, *J. Biol. Chem.*, 1930, **88**, 545; Liang and Noller, *J. Amer. Chem. Soc.*, 1935, **57**, 525), and depressions in m. p. were not observed with mixtures of the two genins and of their benzoates. A distinct depression was, however, produced in a mixture of the acetates and the rotation of nitogenin is roughly twice as high as that of tigogenin, so they are evidently not identical.

## Experimental.

4·5 Kg. of " dates " yielded 530 g. of kernels, which were crushed, freed from fat by extraction with petroleum, re-ground, and divided into three portions, each of which was extracted (Soxhlet) with 500 c.c. of absolute alcohol, the extract being poured into ether (3 vols.). The crude saponin was precipitated as a glue-like mass, but a portion was obtained as a flocculent precipitate which became solid on grinding with ether (*A*). The total amount of crude saponin obtained was 79 g., but contained a good deal of occluded solvent. The crude saponin was deliquescent, but the purer material (*A*) could be kept for a considerable time in an atmosphere of nitrogen; this material had the composition required for a tetraglucoside of a $C_{27}$ sapogenin, although no great significance can be attached to the analytical figures (Found: C, 56·4; H, 7·9. $C_{51}H_{86}O_{24}$ requires C, 56·5; H, 8·0%). The saponin formed a small amount of precipitate with an alcoholic solution of cholesterol.

The crude saponin (52 g.) was boiled with 200 c.c. of water and 20 c.c. of hydrochloric acid for 5 hours. A solid soon began to separate and was colourless at first, but soon darkened. It was collected and boiled with successive portions of water until coloured impurities were no longer extraced, 4·9 g. of crude *sapogenin* being obtained after drying at 100°. This material was extracted (Soxhlet) with pure dry ethyl acetate, and the extract evaporated. The coloured gummy residue was dissolved in alcohol and boiled for ½ hour with activated charcoal, and the solution evaporated. The semi-solid mass was rubbed with ether, causing a solid to separate, and a further crop of solid was obtained by evaporating the ethereal mother-liquor and rubbing the residue with acetone, in which the sapogenin was sparingly soluble. It was then repeatedly crystallised from methyl alcohol, forming small needles, m. p. 201°; it could also be recrystallised from ethyl acetate and was very soluble in chloroform, $[\alpha]_D$ − 112° ($c$ = 0·3918 in chloroform); it gave a cherry-red colour in the Liebermann test and a reddish violet in the Liebermann–Burchard test (Found: C, 77·6; H, 10·5; *M*, Rast, 392. $C_{27}H_{44}O_3$ requires C, 77·8; H, 10·7%; *M*, 416).

The *acetate* was prepared by boiling nitogenin with acetic anhydride and sodium acetate for 3 hours and pouring the solution into water; it crystallised from dilute acetone or methyl alcohol in fine flattened needles, m. p. 191—192° (Found: C, 76·1; H, 10·0. $C_{29}H_{46}O_4$ requires C, 75·9; H, 10·1%). The *benzoate* was prepared in pyridine solution and formed small plates, m. p. 229°, from ethyl acetate or acetone (Found: C, 78·2; H, 9·1. $C_{34}H_{48}O_4$ requires C, 78·4; H, 9·1%). The *o*-bromobenzoate had m. p. 222—223°.

The sugars formed by the hydrolysis of the saponin were recovered by neutralising the mother-liquors from the preparation of nitogenin with barium carbonate and evaporation, but were not obtained in a crystalline condition. They were dextrorotatory ($[\alpha]_D$ + 19° in water), reduced Tollens' reagent in the cold and Fehling's solution on boiling, and gave a red colour in the aniline acetate test. Pentoses were absent, as no colour was produced on distilling the sugars with 12% sulphuric acid and testing the distillate with resorcinol. The only solid derivative obtained so far is glucosazone.

Professor J. H. Gaddum of the College of the Pharmaceutical Society kindly undertook the examination of the physiological properties of the saponin and reports as follows: " The saponin was found to be an active hæmolytic agent. Human blood was collected in 0·2% sodium citrate solution, and the cells washed with 0·9% sodium chloride solution. A volume of 1 c.c. of the cells was suspended in 40 c.c. of saline and 0·2 c.c. of this suspension was mixed with 0·8 c.c. of solutions of the saponin in saline. Tested in this way, the saponin completely hæmolysed the cells in 2·5 minutes when the final concentration was $10^{-5}$. The curve connecting the concentration of saponin and the time for complete hæmolysis was of the usual type.

" The toxicity for tadpoles was similar to that of digitonin (B.D.H.), but death occurred more slowly. Thus concentrations of $10^{-5}$ of the saponin and digitonin caused death in 4·5 and 2 hours respectively. At a concentration of $10^{-4}$ the times were 135 and 17 minutes. At a concentration of $10^{-6}$ the tadpoles survived more than 24 hours.

" A dose of 1 mg. injected subcutaneously in a frog had no apparent effect. The saponin arrested isolated hearts from frogs in much the same way as digitalis, when used in a concentration of $10^{-3}$; this is between 20 and 200 times the concentration of the different digitalis glucosides which produce the same effect. Digitonin is said to be inactive on the heart, but the sample of B.D.H. digitonin produced the same effect in slightly lower concentrations than the *Balanites* saponin. These observations indicate that the digitalis activity of the saponin is so slight as to be negligible."

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