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NOTE

Chemical Investigation of Fresh Roots of *Balanites roxburghii* (Planch)

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The diosgenin glucoside is isolated from fresh roots of *Balanites roxburghii* and its structure established on the basis of NMR and mass spectral data.

Balanites roxburghii commonly known as Hingan or Hingot is an important drug in the indigenous system of treatment.[1–5] The bark, unripe fruits and leaves are considered to be purgative and anthelmintic. The seeds are reported to be expectorant and given in cough and colic. The bark is used as anthelmintic for cattle and the juice of the bark has fish poison properties. During the past 15 years, this plant has been reported to contain spermicidal,[6] antibacterial[7] and cardiovascular activity[8] but later was found insignificant.

The interest in the genus 'Balanites' was generated as a potential source of diosgenin used in oral contraceptives.

The fruits[9–11] seeds[4, 9, 12], leaves[9] and stem[9, 12, 13] have been chemically examined but no work seems to have been done on fresh roots of the plant.

Fresh roots (2 kg) of the wildly growing *B. roxburghii* were collected from the campus of Hamdard Nagar and cut into small pieces. The material was then exhaustively extracted with boiling ethanol. The alcoholic concentrate (15 g) was then successively extracted with petroleum ether and acetone. The insoluble residue left after petrol and acetone extraction was taken up in water and filtered. The filtrate was hydrolysed by dil. H_2SO_4 under boiling condition. An insoluble product which separated out was filtered, washed with water and dried (13 g)

The crude product (10 g) was acetylated with acetic anhydride and pyridine in the cold to give an acetyl derivative (10.5 g) which was extracted with cold methanol several times. The methanolic extract was concentrated to a small volume and extracted with petroleum ether. The petroleum ether extract was evaporated to dryness and crystallised from ethanol to give a compound (A) (1.2 g), m.p. 205°C which was TLC pure on a silica gel G plate C_6H_6 : $CHCl_3$: MeOH, 75 : 25 : 5).

The mother liquor was evaporated to dryness and chromatographed on a column of silica gel. Elution of the column with petroleum ether-benzene gave compound B.

Characterisation of Compound A

It gave a positive LB test. The NMR and mass spectra suggested the compound to be a steroidal glycoside. A few mg of the compound were hydrolysed by Kiliani mixture. The hydrolysate on paper chromatographic examination along with different samples of sugars in the solvent system (butanol : ethanol : water, 4 : 1 : 0.9) showed the presence of glucose only.

The NMR spectrum of the compound revealed the presence of two tertiary methyl groups at δ 0.78 and δ 0.996 and two secondary methyl groups at δ 1.06 and δ0.96 reminiscent of steroidal sapogenins. There were four acetoxyl groups at δ 0.99, δ 2.01, δ 2.04 and δ 2.06 suggesting the compound to be a monoglycoside of a monohydroxy steroidal sapogenin. By comparison of the data available on steroidal sapogenins, the hydrogens on carbon carrying oxygen namely H-26α, H-26β, H-16, H-3 could be located. Thus the H-26α was located at δ 3.27 and the H-26β at δ 3.92. The H-16 could be located at δ 3.48 while H-3 appeared as multiplet around δ 4.4. The olefinic proton at C-6 was found as triplet at δ 5.06. The remaining signals could be accounted for by the protons of the sugar moiety. Thus the multiplet centred at δ 3.66 could be ascribed to C-5′H and the two double doublets centred at δ 4.1 and δ 4.25 could be assigned to the methylenic hydrogens. The anomeric hydrogen appeared as a doublet at δ 5.35 with a very small coupling constant of the order of J = 2 Hz indicating that the sugar is attached by α linkage. Hydrogen at 2′ as a double doublet centred at δ 4.94. The C-3′H′ appeared as a clean triplet centred at δ 5.19 with J = 9.5 Hz coupling which shows that C-3′H is diaxially coupled to two neighbouring hydrogens. Thus these data indicate that the compound is an δ glucoside of diosgenin.

The above data were further supported by mass spectral studies. The mass spectrum of the compound showed the molecular ion peak at m/z 414. Other important peaks, characteristic of diosgnin, could be located at m/z 300, 271 and 139.

The compound 'A' on deacetylation with 5% methanolic KOH gave a compound with m.p. 221°C, which was identified as diosgenin on m.m.p., Co TLC examination and superimposible IR spectrum.

Hence the compound A was identified as diosgenin glucoside tetraacetate and the parent compound was identified as diosgenin glucoside. Since this compound has been obtained on hydrolysis of the saponin, it is likely that this compound is the prosapogenin of the saponin of diosgenin. The aglycone from the hydrolysate was identified as diosgenin by direct comparison with an authentic sample.

Characterisation of Compound 'B'

The compound 'B' on deacetylation gave a compound which on the basis of its physical data, m.m.p., on TLC examination and superimposable IR spectra was identified as diosgenin.

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