TRITERPENOIDS I. THE SAPOGENINS OF FAGONIA CRETICA LINN. (ZYGOPHYLLACEAE)

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The water soluble saponins, which are present in *Fagonia cretica* in high concentration, have been extracted and hydrolysed into a mixture of sapogenins and sugars. Among the five sugars found in the hydrolysate, four have been identified as glucose, rhamnose, xylose and arabinose. From the mixture of sapogenins, two pure, well-crystalline sapogenins, provisionally named as Fagogenin and Genin-A, have been isolated. The molecular formulae of Fagogenin and Genin-A have been found to be $C_{30}H_{48}O_4$ and $C_{29}H_{46}O_5$ respectively and the former has been characterised by preparation of its diacetate and dibenzoate. Spectroscopic studies on both the compounds have been made and the oxygen functions of Fagogenin have been determined. Fagogenin has been found to contain a lactone and two hydroxyl groups in addition to, at least, one double bond.

Fagonia cretica Linn., a small green spiny undershrub which grows throughout North Western India, Sind, the Punjab, and the southern provinces of the Western Peninsula, is reputed in the indigenous system of medicine as a tonic, febrifuge and prophylactic against smallpox.[1] It has also been used in the treatment of dropsy. As literature search showed that no chemical work has hitherto been done on this plant, and also because its aqueous extract has been recently claimed by some of the physicians of the Unani Tib as useful in the treatment of certain types of cancer, it was considered of interest to undertake a chemical investigation of the plant.

As a result of the present investigations, it has been found that the plant contains water soluble saponins in high concentration. On hydrolysis with dilute acid, the saponins yielded a crude mixture of sapogenins, the major portion (*ca.* 60%) of which was alkali soluble, thus indicating the presence of one or more acid sapogenins in the mixture. From the neutral portion, we have isolated a pure crystalline sapogenin which we have named provisionally "Fagogenin".

Fagogenin, $C_{30}H_{48}O_4$, m. p. 307-308°, $[\alpha]_D^{28}$ +26.36°, crystallised in white glistening needles from ethanol, methanol-petroleum ether, but best from benzene. It gave with the Liebermann-Burchard reagent a deep red colour. With tetranitromethane it did not give any colour nor did it show any selective absorption in the ultraviolet region above 210 mμ. The infra-red spectrum of Fagogenin in $CHCl_3$ solution showed strong bands at 3571 cm^{-1} (hydroxyl) and 1730 $cm.^{-1}$ (carbonyl) while that in KBr (Fig. 1) showed the corresponding peaks at 3472 cm^{-1} and 1718 cm^{-1}.

With acetic anhydride in pyridine at room temperature, Fagogenin gave a diacetate, $C_{34}H_{52}O_6$, which crystallised from ethanol-water or, better, from petroleum ether in the from of colourless, glistening needles, m. p. 243°, $[\alpha]_D^{27}$ + 50.10°. The infra-red spectrum of the diacetate in KBr showed strong bands at 1733 cm^{-1} (with shoulder at 1742 cm^{-1}) and 1248 cm^{-1} (ester) but no OH absorption. Whilst Fagogenin itself gave no colour with tetranitromethane, the diacetate gave a strong yellow colour with this reagent indicating the presence of a carbon-carbon double bond which is evidently of the unreactive type present in triterpenes.[2]

With benzoyl chloride in presence of pyridine, Fagogenin yielded a dibenzoate, $C_{44}H_{56}O_6$, which crystallised from benzene-petroleum ether in the form of clusters of white leaflets, m.p. 264-5°, $[\alpha]_D^{28}$ + 100.62°. The infra-red spectrum of the dibenzoate in KBr showed a doublet at 1582 and 1600 $cm.^{-1}$ (aromatic) and strong bands at 1733 (C=O of benzoate), 1712, 1267 and 1110 cm^{-1}. The last two bands are due to C-O-stretching vibration of the C_6H_5COO-group.

The formation of the diacetate and the dibenzoate and, at the same time, the absence of any OH absorption in their infra-red spectra, prove that Fagogenin contains only two hydroxyl groups.

Fagogenin did not give an oxime, a semicarbazone or a D.N.P. derivative even on refluxing with the ketonic reagents for 2-3 hours. On refluxing with 10 percent ethanolic potash for 3 hours, neither did it produce any acid. But when it was refluxed with 10 percent KOH in diethylene glycol for 3 hours, an acid was obtained in a very low yield proving that Fagogenin contained an ester

or lactone group that was very resistant to hydrolysis. The acid crystallised from ethanol in the form of very fine, white needles, m.p. > 300° (decomp.) and its infra-red spectrum in KBr (Fig. 3) was found to be very similar to that of Fagogenin except that a new broad band appeared at 2551 cm.$^{-1}$ (carboxylic OH)[3] and the only carbonyl absorption was at 1695 cm.$^{-1}$ (C=O of carboxyl) instead of 1718 cm.$^{-1}$. With diazomethane the acid formed a methyl ester which crystallised from methanol in clusters of fine, long needles, m.p. 224-5°. Infra-red spectrum of the methyl ester in KBr was clearly distinct from that of Fagogenin and showed strong absorption bands at 3278 cm.$^{-1}$, 1724 cm.$^{-1}$ and 1197 cm.$^{-1}$ (ester).[4] It appears, therefore, that Fagogenin is a dihydroxy lactone belonging to the triterpenoid series.

While working in a different procedure than that adopted in the isolation of Fagogenin, another crystalline substance was obtained in a comparatively much lower yield than Fagogenin. This substance crystallised from ethanol in long, white needles that melted at 326-27°. We have tentatively designated this substance as Genin-A. Combustion analysis of Genin-A gave values which

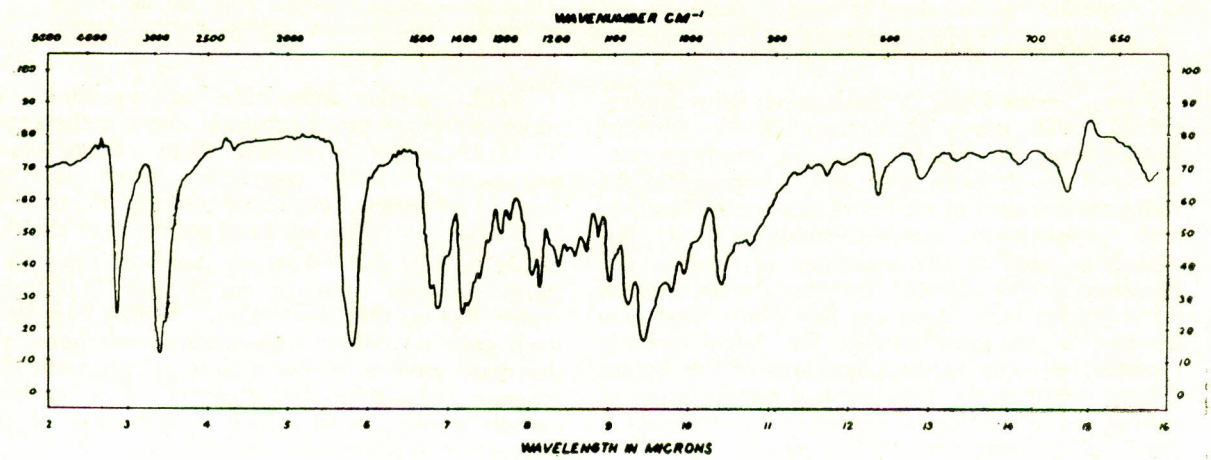

Fig. 1.—Infra-red spectrum of Fagogenin in KBr.

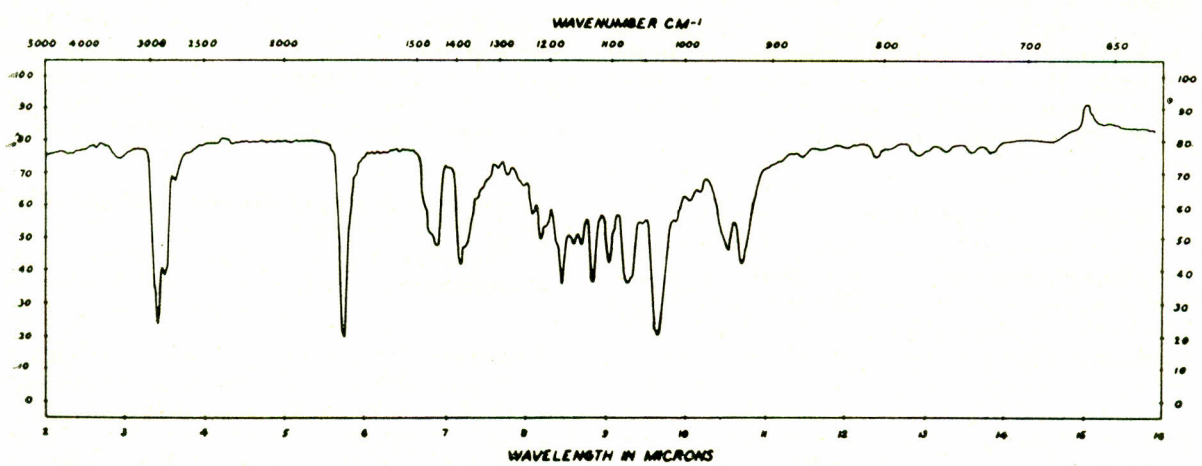

Fig. 2.—Infra-red spectrum of Genin-A in KBr.

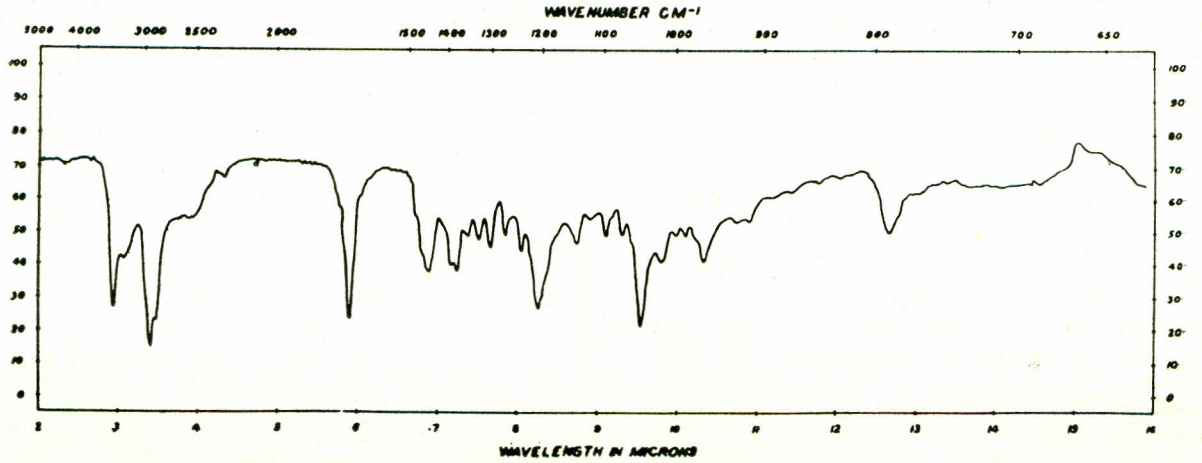

Fig. 3. Infra-red spectrum of the acid, obtained on alkaline hydrolysis of Fagogenin, in KBr.

are consistent with the molecular formula $C_{29}H_{46}O_5$. Genin-A gave a yellow colour with tetranitromethane and with the Liebermann-Burchard reagent a light purple. Its infra-red spectrum in KBr (Fig. 2) showed a carbonyl absorption band at 1748 cm.$^{-1}$ but no hydroxyl.

Experimental

The melting points above 300°C. were taken with the Gallenkamp electric melting point apparatus. Infra-red spectra were determined with the Beckman IR5 spectrophotometer. Combustion analyses were done by Dr. R. A. Shah of Micro-analytical Section, Central Laboratories, P. C. S. I. R. Merck's alumina was used in adsorption chromatography.

Extraction of the Plant.—The aerial parts of the fresh, mature plants (5 kg.), which were collected in Karachi, were cut into small pieces and soaked in rectified spirit (14 litres). The extract was taken out after ten days and concentrated to *ca.* 2 litres (5 ml. octyl alcohol being added to the extract to prevent formation of foams). The remaining solvent was distilled off under reduced pressure and the dark coloured semi-solid residue (298 g.) was taken up in water (*ca.* 2 litres). The aqueous portion was shaken a few times with pet. ether and then once with ether (1 litre) and, finally, with chloroform (1 litre). In all the cases hard emulsions were formed that took one or two days to break except in the last case where an addition of a small amount of alcohol was found to be essential. The petroleum ether-soluble portion yielded a few pure, crystalline compounds which will form the subject matter of next communication.

The aqueous layer was found to contain a large amount of saponins as it formed a lot of stable foams on shaking and, at the same time, haemolysed red blood corpuscles. It gave precipitates with sodium hydroxide and lead acetate solutions but not with the usual alkaloidal reagents. With Fehling's solution, it gave a positive test for reducing sugars.

Hydrolysis of Saponins.—The aqueous solution of saponins (300 ml.) was made approximately 1N by adding concentrated hydrochloric acid (30 ml.) and the mixture boiled under reflux for about 1½ hour when a dark solid mixture of sapogenins separated which was filtered, washed thoroughly with water and then dried in an oven at 140°. The aqueous filtrate was passed through a column of Amberlite resin MB-1 until the eluate was free from chloride ions (no precipitate with $AgNO_3$ solution). The eluate was evaporated to dryness under reduced pressure when a syrupy mass was obtained as residue which was chromatographed on paper (Whatman No. 1) by ascending method using the solvent mixture n-butanol (10)/pyridine (3)/water (3) for elution.[5] On spraying with aniline oxalate, five spots were observed whose R_{xylose} values were 0.56, 0.74, 0.89, 1.00 and 1.38. The spots with R_{xylose} 0.74, 0.89 and 1.00 were pink and they were due to arabinose, an unidentifiable pentose and xylose respectively. The spots with R_{xylose} 0.56 and 1.38 were brown and they were due to glucose and rhamnose respectively. A control run with a mixture of arabinose, xylose, glucose and rhamnose showed the same R_{xylose} values.

Isolation of Fagogenin.—The dried, crude mixture of sapogenins (6.65 g.) was finely powdered and treated with 5% NaOH solution, the mixture

slightly warmed and filtered. This process was repeated once again with the residue which was afterwards thoroughly washed with water. The neutral residue (2.86 g.) thus obtained was dried, powdered, treated with benzene at room temperature and filtered. The filtrate, on removal of solvent, yielded a cream-coloured semi-crystalline powder (A; 0.93 g.). The benzene insoluble residue was dried, powdered and extracted with hot chloroform. The chloroform solution, on removal of solvent, yielded a brown, semi-crystalline powder (B; 1.19 g.). The insoluble residue was again dried and extracted in the same manner with methanol at room temperature. The methanol solution, on drying, yielded a dark coloured mass (0.63 g.). The residue was rejected.

(A) was dissolved in chloroform and chromatographed on a column of acid-washed alumina (in benzene). The column was first eluted with benzene, then with a mixture of benzene-chloroform (gradually increasing the proportion of chloroform) and, finally, with pure chloroform. All the eluates obtained with pure benzene and benzene-chloroform mixtures yielded oils (total weight 0.402 g.) which could not be crystallised. The chloroform eluates yielded a white solid (0.298 g.) which crystallised from methanol-petroleum ether in the form of white needles which, on recrystallisation from benzene, gave needles, m.p., 307-8°, $[\alpha]_D^{28}$ +26.36° (c=1.138, $CHCl_3$), (Found: C, 76.45; H, 10.36%. $C_{30}H_{48}O_4$ requires: C, 76.22; H, 10.24%). This crystalline sapogenin has been named 'Fagogenin'. Fagogenin was found to be highly soluble in methanol and acetone, sparingly soluble in ether and benzene, and insoluble in petroleum ether. Its infra-red spectrum in KBr (Fig. 1) showed absorptions at 3472s, 1718s, 1237m, 1221m, 1106m, 1077m, 1055s, 1018m, 1003m and 957m cm.$^{-1}$. The infra-red spectrum of Fagogenin in chloroform solution showed the carbonyl and hydroxyl absorptions at 1730 and 3571 cm.$^{-1}$ respectively. Fagogenin did not show any ultraviolet absorption maximum above 210 mμ.

(B) was dissolved in hot benzene, concentrated and adsorbed on the top of an acid-washed alumina column. It was then eluted successively with benzene, benzene-chloroform (gradually increasing the proportion of chloroform) and finally with pure chloroform. The eluates obtained with benzene and chloroform-benzene (1:9) yielded some more Fagogenin (0.238 g.).

Chromatography of the fraction (C) on alumina column did not give any crystalline matter.

Fagogenin gave a deep red colour with Liebermann-Burchard reagent. Addition of tetranitromethane to a chloroformic solution of Fagogenin did not produce any colour.

Isolation of Genin-A.—The crude mixture of sapogenins (11.6 g.) was powdered and repeatedly extracted with boiling ether. The extract, on removal of solvent, yielded a brown semi-crystalline powder (6.32 g.) which was again extracted a few times with boiling petroleum ether and the combined extracts filtered hot. The filtrate, on removal of solvent, yielded a brown, semi-crystalline solid (2.36 g.) which was dissolved in benzene and chromatographed on a column of alumina. Elution was first done with benzene, then with chloroform-benzene (1:19 and 1:9) and, finally, with pure chloroform. Eluates with pure benzene and pure chloroform gave oils which did not crystallise whereas those with chloroform-benzene mixtures yielded solid residues which crystallised from ethanol in the form of long, shining needles, m.p. 326-27° (Found:C, 73.13, 73.53; H, 10.19, 9.57%. $C_{29}H_{46}O_5$ requires: C, 73.38; H, 9.77%). This compound has been tentatively designated as Genin-A. On addition of tetranitromethane to a solution of Genin-A in chloroform, a yellow colour was produced. With Liebermann-Burchard reagent it gave a light purple colour. Infra-red absorption spectrum of Genin-A in KBr (Fig. 2): $\nu_{max.}$ 1739s, 1180m, 1228m, 1105m, 1075m, 1033s, 946m and 932m cm.$^{-1}$

Fagogenin Diacetate.—Fagogenin (250 mg.), acetic anhydride (2.5 ml.) and pyridine (2 ml.) were mixed and left overnight at room temperature. Next day, the white solid that separated on treatment with water, was extracted with ether and worked up in the usual procedure. The diacetate crystallised from petroleum ether in the form of colourless, glistening needles, m.p., 243°, $[\alpha]_D^{27}$ + 50.10° (c=0.978, $CHCl_3$) (Found: C, 73.30; H, 9.60%. Calc. for $C_{30}H_{46}O_2(OCOCH_3)_2$: C, 73.34; H, 9.41%). The yield was 245 mg. The acetate also crystallises from ethanol-water in needles. Infra-red absorption spectrum in KBr: $\nu_{max.}$ 1742 (shoulder), 1733s, 1470m, 1248s and 1038s cm.$^{-1}$.

A chloroformic solution of the diacetate produced a strong yellow colour with tetranitromethane. The Liebermann-Burchard test was negative.

Fagogenin Dibenzoate.—Benzoyl chloride (3.5 ml.) was added to a solution of Fagogenin (442 mg.) in pyridine (2.5 ml.) and the mixture, after standing for four days at room temperature, was

treated with excess of water. The oily mass was extracted with ether and the extract shaken with dilute hydrochloric acid, water, cadmium chloride, $NaHCO_3$ and, finally, with water. After drying (Na_2SO_4), the solvent was removed when a yellow oil was obtained which could not be crystallised.

A solution of the oil in benzene was adsorbed at the top of an acid-washed alumina column (in petroleum ether) and chromatographed by eluting, at first, with petroleum ether, then with a mixture of benzene-petroleum ether (gradually increasing the proportion of benzene) and, finally, with pure benzene. All the fractions obtained on elution with benzene-petroleum ether and pure benzene, yielded pale yellow oily residues which crystallised from benzene-petroleum ether. The maximum amount of crystalline substance was obtained from the 10 percent benzene- 90 percent petroleum ether eluate. All the crystalline substances were combined and finally recrystallised from the same solvent when the benzoate was obtained in the form of clusters of white leaflets, m.p. 264-5°, $[\alpha]_D^{28}+100.62°$ (c=1.113, $CHCl_3$) (Found: C, 77.14, 77.78; H, 7.95, 8.21%. Calc. for $C_{30}H_{46}O_2(OCOC_6H_5)_2$: C, 77.61; H, 8.29%). The yield was 362 mg. The infra-red absorption spectrum of the benzoate in KBr: ν max. 1733s, 1712s, 1600w, 1582w, 1315m, 1267s, 1179m, 1110s, 1072m, 1026m and 718s cm.$^{-1}$. A solution of the dibenzoate in chloroform did not produce even a weak yellow colour with tetranitromethane.

Hydrolysis of Fagogenin.—Fagogenin (200 mg.) was added to a 10 percent solution of KOH in diethylene glycol (25 ml.) and the mixture gently refluxed for 3 hours. After this period, the solvent was distilled off under reduced pressure, the residual brown hydrolysate treated with water and filtered. The aqueous filtrate was made acidic when a small amount of brown precipitate was obtained. It was centrifuged out and found to be too small for investigation. The residue was extracted thrice with boiling ethanol (10 ml. each time) and the combined ethanolic extracts, on concentration, spontaneously crystallised. The solid, on recrystallisation from ethanol, gave very fine, white needles which melted above 300° with decomposition. Its infra-red absorption spectrum showed it to be an acid. IR spectrum in KBr (Fig. 3): ν max. 3367s, 3226m, 2551w, 1695s, 1207s, 1043s, 1016m, 966m and 788w cm.$^{-1}$.

Ethereal diazomethane was added to an ethanolic solution of the acid and the mixture left overnight. Next day, the solvents were carefully removed and the residue crystallised from methanol in clusters of fine, long needles, m.p. 224-5°. Infra-red spectrum of the methyl ester in KBr showed absorptions at 3278s, 1724s, 1197s and 978m cm.$^{-1}$.

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