

Mass Spectrum:  $m/e = 594 (M^+, 100\%), 593 (53\%),$   
 $382 (46\%), 381 (85\%), 368 (8\%), 367 (40\%), 364 (8\%),$   
 $297 (3\%), 192 (17\%), 191 (75\%), 174 (17\%), 168 (14\%).$

3.1

I N T R O D U C T I O N

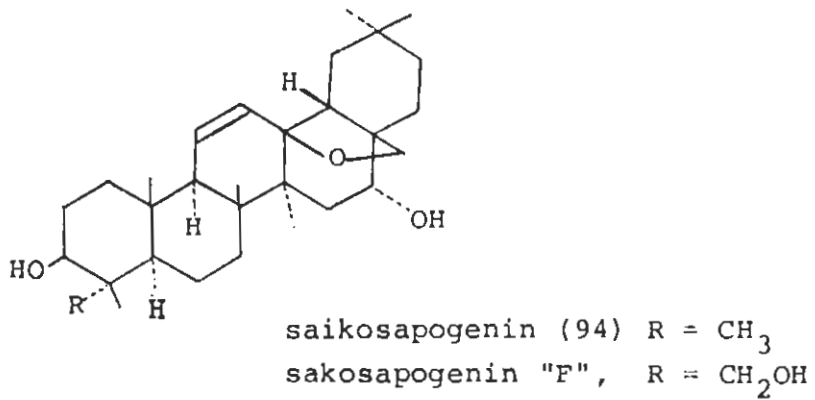
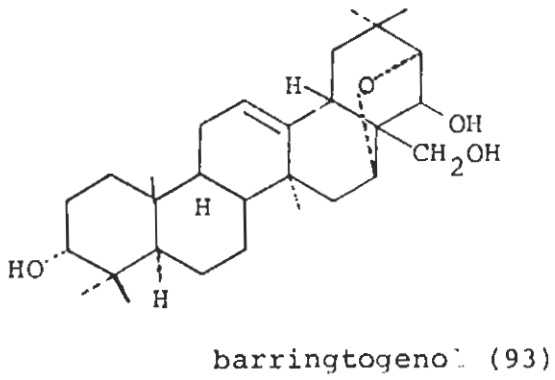
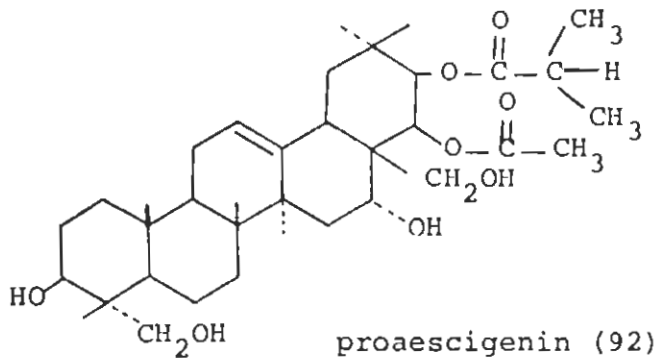
*Fagonia indica* Linn<sup>139</sup> (*Fagonia cretica*), family Zygophyllaceae is a small spiny undershrub with stiff branches often more or less prostrate. It has tiny rose-coloured solitary flowers found almost all the year round. It is a very common plant, widely distributed throughout Pakistan. The aqueous decoction of leaves and young twigs is a popular remedy for the treatment of skin lesions (boils and abscesses) particularly amongst children. It is described as astringent and a cure for any disorders arising from poisoning.<sup>140</sup> It is reputed in the indigenous system of medicine as a tonic, febrifuge and prophylactic against smallpox.<sup>140</sup> It has also been used in the treatment of dropsy. Its aqueous extract has been claimed by some physicians of "Unani Tibb" as being useful in the treatment of certain types of cancer. Preliminary pharmacological tests of aqueous extracts on mice have shown some anticancer activity.<sup>141</sup> An Ames mutagenicity test has also indicated marginal activity.<sup>142</sup> The plant contains water-soluble and water-insoluble saponins,<sup>143</sup> which are recognized by their characteristic properties such as foaming, haemolysis,

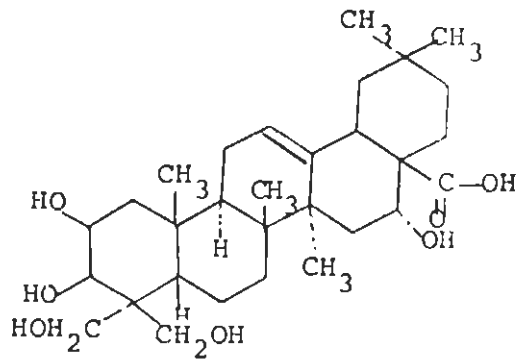
and behaviour as fish poison etc. Plant drugs containing saponins are often used as expectorant and anti-tussive agents.

Saponins are oligoglycosides of triterpenoids or steroids and are chemically classified into monodesmosides and bisdesmosides. The former bear a single sugar chain which is attached to the aglycone unit with a glycosidic linkage while the latter contain two sugar chains which are linked at two different positions of the aglycone with glycosidic or ester linkages. Steroidal saponins are known to be more important since they are used as the starting materials for the synthesis of steroid hormones and related chemotherapeutic agents. Some triterpenoid saponins have been shown to possess interesting biological activities. Recently the pharmacological activities of saponins have been extensively studied with the object of developing new therapeutic agents.<sup>144</sup> Brief mention may be made here of a number of other important saponin containing plants.

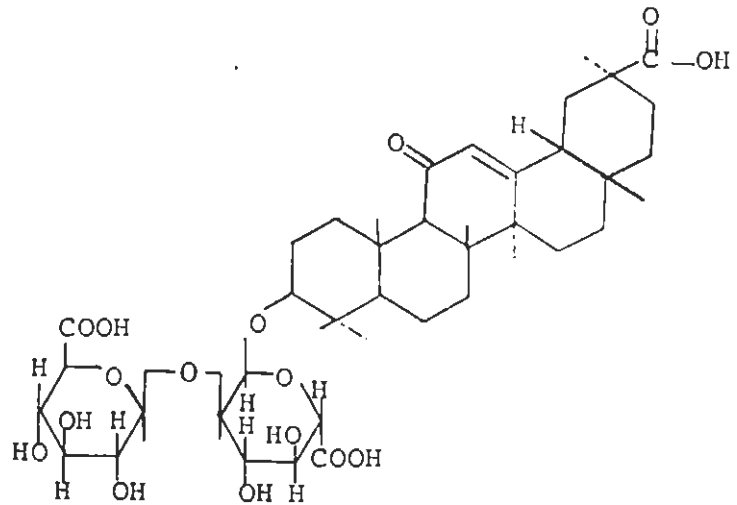
In Western folk medicine, the seeds of *Aesculus hippocastanum* L. (Hippocastaceae) have been used for the treatment of haemoloid and venous congestion. The saponins

of the seeds, named "aescin", was found by Wulff and Tschesche<sup>145</sup> to be a mixture of oligoglycosides of pro-aescigenin (92) and baringtogenol (93) and employed clinically by oral administration. Saponins are also found to occur in several traditional drugs used in Chinese medicine. Thus the root of *Bupleurum falcatum* L. (umbelliferae) has been used under the name of "chai-fu" as a major component of several prescriptions to resolve the tightness and resistance syndrome at the costal margin that might be related to inflammation and disorder of liver. The main constituents of this root were recognised as oligoglycosides of saikosapogenin a,c and d (94) isolated by Kubota and his co-workers.<sup>146</sup> The root of *Platycodon grandiflorum* DC (complanulaceae) has also been used under the name of "chick-keng" in Chinese medicine as expectorant and an antitussive agent. It contains saponins which produce platycodigenin (95) as the major sapogenin on acid hydrolysis.<sup>147</sup> Another important saponin containing plant drug is "licorice". This root extract of *Glycyrrhiza* spp. is a well known plant drug in both Western and oriental medicines. The extracts of the root are used as sweetening, expectorant and anti-tussive agents since the ancient Greek times. In Chinese medicine licorice is contained as a component in many





platycodigenin (95)



glycyrrhizin (96)

prescriptions, acting as a spasmolytic agent, a calmative and an antidote. The main principle of licorice is a sweet tasting saponin glycyrrhizin (96) which on acid hydrolysis give an oleanane-type triterpene, glycyrrhetic acid.<sup>148,149</sup> It has been reported by several workers<sup>150</sup> that glycyrrhizin and glycyrrhetic acid revealed anti-ulcer<sup>150</sup> and anti-allergic actions.<sup>151,152</sup>

Investigation of the chemical constituents of *Fagonia cretica* (*F.indica*) carried out by K.P.Tiwari and co-workers<sup>143</sup> led to the isolation of  $\beta$ -sitosterol, n-triacentanol and ceryl alcohol. In 1966 Wasif Hussain and co-workers<sup>153</sup> reported that the acetone soluble fraction of the alcoholic extracts contain chinovic acid. In 1966 Amjad Ali and co-workers hydrolysed the water-soluble saponins and obtained four sugars namely glucose, rhamnose, xylose and arabinose and two sapogenins which they named "Fagogenin" ( $C_{30}H_{48}O_4$ ) and "genin A" ( $C_{30}H_{50}O_2$ ).<sup>154</sup> In 1967 the same authors reported the isolation and identification of "fagogenin", Genin A and Genin B ( $C_{30}H_{50}O_2$ ) from the neutral sapogenin fraction of *Fagonia cretica*.<sup>155</sup> Ahmad Rizk and co-workers<sup>156</sup> have reported the presence of lipids in six Egyptian *Fagonia* species. The lipid content was found to vary between 0.3-1.14%. They also

reported the presence of  $\beta$ -sitosterol and campesterol. In 1969 this group communicated the isolation of aglycone "B", fagonin, and oleanolic acid from the sapogenin fraction of *Fagonia cretica*.<sup>157</sup> The same group reported the isolation of oleanolic acid, betulic acid and fagonin.<sup>158</sup>

Z.F.Ahmed and co-workers<sup>159</sup> have studied six different species of *Fagonia indica* and identified two free sugars by paper chromatography, namely glucose and maltose. They also isolated aglycone "A", fagonin ( $C_{25}H_{16}O_2$ ) m.p. 152-154°C and aglycone "B" m.p. 280-283°C from the hydrolysed saponin fraction. The sugars isolated from this fraction, were identified by paper chromatography as glucose, arabinose and rhamnose. They have also reported the isolation of harman ( $C_{10}H_{12}N_2$ ) from the plant.<sup>160</sup> D.V.Zaitschek and co-workers<sup>161</sup> obtained oleanolic acid as a sapogenin. Iyer and co-workers<sup>162</sup> have described the isolation of two unidentified compounds ( $C_{15}H_{29-30}O$ ) three triterpenoids  $C_{30}H_{40}O_4$ ,  $C_{30}H_{48}O_5$ ,  $C_{30}H_{46}O_5$  and harmine from *Fagonia indica*. M.Saeed Arayne and co-workers<sup>163</sup> have reported the presence of fatty acid in *Fagonia indica*. In 1979 A.M.Khan and co-workers<sup>164</sup> have reported the isolation and identification of several

amino acids from *Fagonia indica* namely alanine, arginine, glycine, isoleucine, leucine, lysine, phenyalanine, proline, tyrosine and valine.

### 3.2 RESULTS AND DISCUSSION

Many plants belonging to the family Zygophyllaceae have been used in the indigenous system of medicine for the treatment of various diseases. The plant *Fagonia indica* (Zygophyllaceae) is reputed as a medicinal plant in scientific and folkloric literature but only a limited chemical investigation has been carried out on it.

In this section of the thesis the isolation and structure elucidation of a new sapogenin, new saponins and pinatol from *Fagonia indica* Linn<sup>139</sup> is presented. As a result of the present work three sapogenins, four saponins and pinatol have been isolated.

#### A. ISOLATION AND STRUCTURAL STUDIES ON SAPOGENINS OF FAGONIA INDICA

Keeping in view the importance of using fresh plant materials in indigenous system of medicine, the fresh aerial parts of *Fagonia indica* were collected and crude saponins were isolated by extraction with ethanol. The crude saponins were hydrolysed with 20% ethanolic HCl to afford the corresponding sapogenins which was chromatographed on a silica gel column. The column was packed in

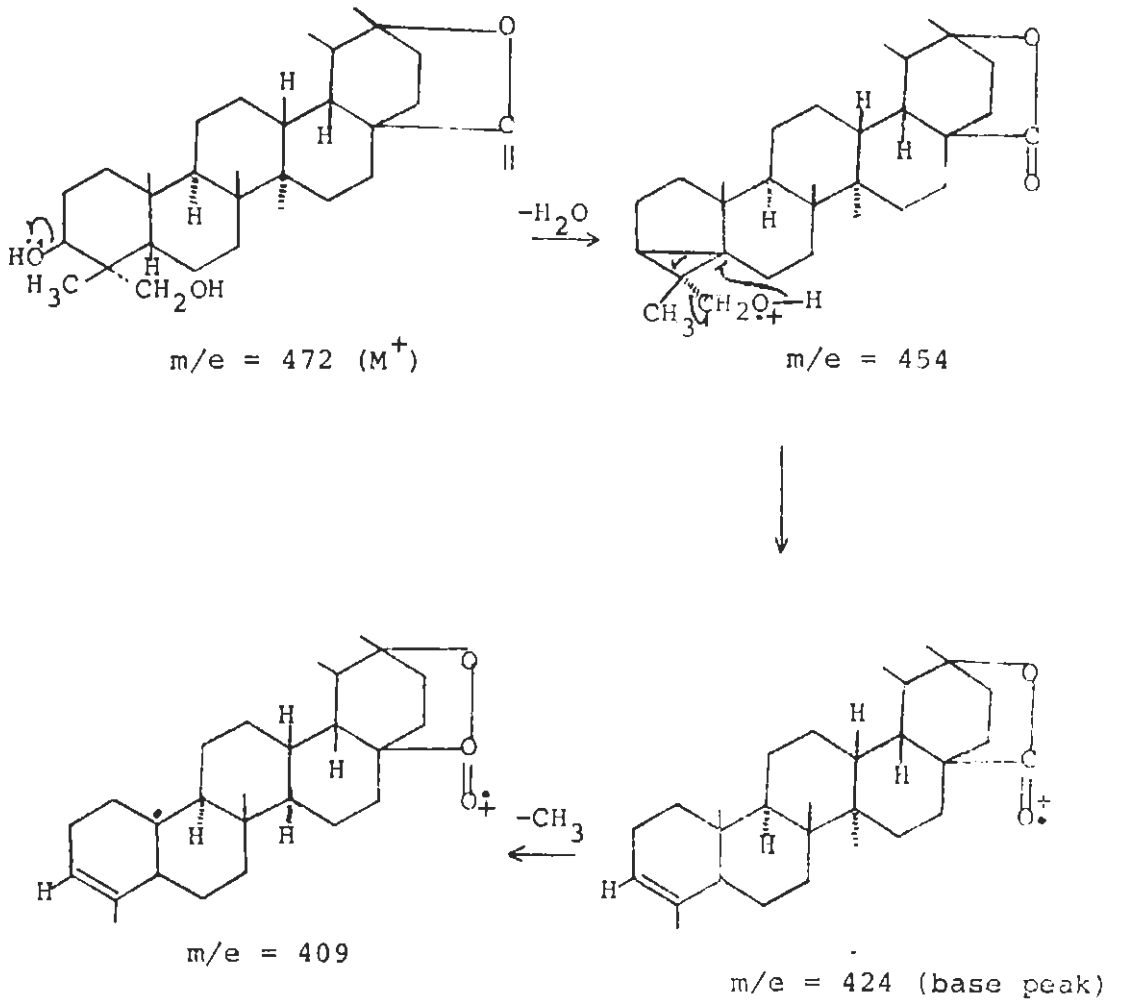
petroleum-ether. Elution was carried out with petroleum-ether, petroleum ether-chloroform, chloroform, chloroform-methanol and finally with methanol. The chloroform-methanol eluates (FG) afforded two crystalline compounds. These were further purified by crystallisation. The first of these was a new sapogenin named "nahagenin"(81). The second was identified as hederagenin (83).

i) Isolation and Structure of Nahagenin (81)

Nahagenin (81) m.p.  $298^{\circ}\text{C}$  gave a positive Leibermann Burchard test for triterpenoids and a red colouration with trichloroacetic acid which indicated it to be a pentacyclic triterpene.<sup>165</sup> Nahagenin (81) analyzed for  $\text{C}_{30}\text{H}_{48}\text{O}_4$  and this formula was confirmed by high resolution mass spectrometry, the molecular ion peak being recorded at  $m/e = 472.3540$  in agreement with the formula  $\text{C}_{30}\text{H}_{48}\text{O}_4$  (requires 472.3552). The I.R. spectrum ( $\text{CHCl}_3$ ) showed absorptions at  $1740\text{ cm}^{-1}$  indicating the presence of a 6-membered lactone. A prominent absorption at  $3460\text{ cm}^{-1}$  indicated the presence of an OH group. The proton magnetic resonance spectrum ( $\text{CDCl}_3$ , 100 MHz) showed the presence of six quaternary methyl groups at  $\delta 0.87$ ,  $\delta 0.88$ ,  $\delta 0.91$ ,  $\delta 0.94$ ,  $\delta 1.14$  and  $\delta 1.36$ , and indicated

the absence of olefinic protons. The  $^{13}\text{C}$ -magnetic resonance spectrum ( $\text{CDCl}_3$ , 100 MHz) confirmed the presence of 30 carbon atoms. The chemical shift of each carbon atom and the substitution as determined by the off-resonance spectrum is assigned in Table-II. The carbon atoms in rings A and B were readily recognised by comparison with signals of other pentacyclic triterpenoids.<sup>166</sup> The six quaternary carbon atoms resonated as singlets at  $\delta$  41.86,  $\delta$  40.51,  $\delta$  37.05,  $\delta$  41.08,  $\delta$  42.06 and  $\delta$  84.27 which were assigned to  $\text{C}_4$ ,  $\text{C}_8$ ,  $\text{C}_{10}$ ,  $\text{C}_{14}$ ,  $\text{C}_{17}$  and  $\text{C}_{20}$  respectively. The six methyl groups resonated as six quartets at  $\delta$  11.30,  $\delta$  15.69,  $\delta$  16.63,  $\delta$  23.98,  $\delta$  14.26 and  $\delta$  23.98, which were assigned to  $\text{C}_{24}$ ,  $\text{C}_{25}$ ,  $\text{C}_{26}$ ,  $\text{C}_{27}$ ,  $\text{C}_{29}$  and  $\text{C}_{30}$  respectively. The  $^{13}\text{C}$ -nuclear magnetic resonance spectrum also indicated the absence of olefinic carbon atoms. One downfield triplet at  $\delta$  41.98 was assigned to  $\text{C}_{21}$  which was attached to the oxygen bearing carbon. The presence of a lactone ring was indicated from the  $^{13}\text{C}$ -nuclear magnetic resonance spectrum which showed an absorption at  $\delta$  177.29 for the lactone carbonyl group. Three downfield absorptions at  $\delta$  84.72,  $\delta$  76.54 and  $\delta$  71.92 were assigned to  $\text{C}_{20}$ ,  $\text{C}_3$  and  $\text{C}_{23}$  carbon atoms respectively. The presence of a singlet at  $\delta$  84.72 indicated that the lactone oxygen was attached to a quaternary center ( $\text{C}_{20}$ ) while

the absorption at  $\delta$  71.92 appeared as a triplet (doublet of doublets?)( $C_{23}$ ). The peak at  $\delta$  76.54 appeared as a doublet which was consistent with its assignment to the hydroxyl bearing carbon atom at  $C_3$ . Other  $^{13}C$ -nuclear magnetic resonance signals were assigned by means of off-resonance techniques and by application of known chemical shift rules.<sup>166</sup> The mass spectrum of nahagenin showed the molecular ion peak at  $m/e = 472 (M^+)$  and other peaks appeared at  $m/e = 454, 436, 424, 409, 396, 395$  and  $261$ . The mass fragments are given in Scheme 32. The substance readily afforded a diacetate in the presence of acetic anhydride and pyridine at room temperature having the molecular ion peak at  $m/e = 556 (M^+)$ . This was consistent with the presence of two acylable -OH groups in nahagenin. The proton nuclear magnetic resonance spectrum of the diacetate again indicated the presence of six quaternary methyl groups at  $\delta$  0.85,  $\delta$  0.89,  $\delta$  0.95,  $\delta$  1.14,  $\delta$  1.16 and  $\delta$  1.36 and the absence of olefinic protons. A singlet appeared at  $\delta$  2.54 corresponding to the methyl protons of the acetyl group. The mass spectrum of the diacetate afforded the molecular ion at  $m/e = 556$  and other major peaks at  $496, 436, 368, 323, 284, 233, 190, 161$  and  $119$ . The substance was found to be remarkably inert



Scheme-32

to attempted hydrolysis of the lactone system. No acid was obtained when nahagenin (81) was refluxed with 10% ethanolic NaOH for 3 hours or with 20% NaOH in diethylene glycol. On the basis of these spectroscopic data structure (81) is assigned to nahagenin. This was confirmed by x-ray diffraction analysis<sup>167</sup> carried out by Prof. Jon Clardy, Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, N.Y 14853, U.S.A.

ii) Isolation and Structure of Hederagenin

The chloroform-methanol fraction (FG) afforded hederagenin which was further purified by crystallisation in methanol. The substance gave positive Liebermann-Burchard test for triterpenes and red colouration with trichloroacetic acid.<sup>165</sup> It melted at 302°C. High resolution mass spectrometry afforded the exact mass of molecular ion  $m/e = 472.3525$  in agreement with the formula  $C_{30}H_{48}O_4$  (requires 472.3552). The I.R. spectrum (KBr disc) showed an absorption at  $3440\text{ cm}^{-1}$  indicating the presence of -OH group. Another absorption at  $1700\text{ cm}^{-1}$  indicating the presence of a COOH group. The proton magnetic resonance spectrum ( $CD_3OD$ , 100 MHz) indicated the presence of six quaternary methyl groups at  $\delta$  0.68,  $\delta$  0.70,  $\delta$  0.90

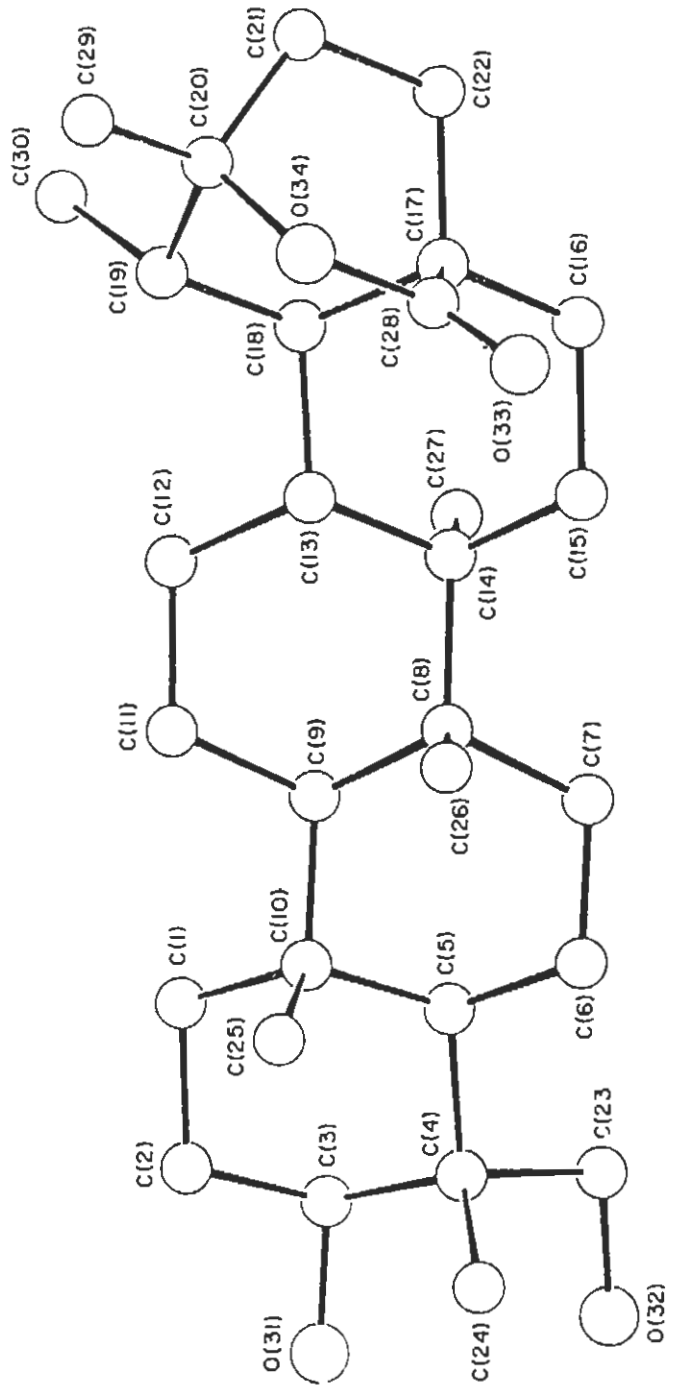


Figure 1. A computer generated perspective drawing of naphaquinin. Hydrogens are omitted for clarity.

$\delta$  0.93,  $\delta$  0.97 and  $\delta$  1.16 and a multiplet at  $\delta$  5.24 (H, m, H-C=C<) for the presence of an olefinic proton.

The mass spectrum of sapogenin II showed the molecular ion peak at  $m/e = 472 (M^+)$  and other peaks appeared at  $m/e = 248, 203, 175, 133$  and  $119$ . On the basis of the above spectroscopic data the sapogenin II was suspected to be hederagenin (83). This was confirmed by direct spectroscopic and chromatographic comparison with an authentic sample. The compound was acetylated at room temperature with acetic anhydride and pyridine when it was converted to the corresponding diacetate exhibiting the molecular ion peak at  $m/e = 556 (M^+)$ . This showed the presence of two acylable -OH groups. The I.R. spectrum ( $CHCl_3$ ) showed absorption at  $1734 \text{ cm}^{-1}$  indicating the presence of ester. The proton magnetic resonance spectrum ( $CDCl_3$ , 60 MHz) showed the presence of six quaternary methyl groups at  $\delta$  0.75,  $\delta$  0.95,  $\delta$  1.25,  $\delta$  1.90,  $\delta$  1.28 and  $\delta$  1.45. A singlet appeared at  $\delta$  2.57 corresponding to the methyl protons of the acetyl group and a multiplet at  $\delta$  5.24 (H, m, H-C=C<) due to the olefinic proton. The mass spectrum of the diacetate afforded the molecular ion peak at  $m/e = 556$  and other peaks appeared at  $m/e = 496, 436, 248, 203, 149$  &  $95$ .

Esterification of the sapogenin II with diazomethane afforded the methylester. The I.R. spectrum ( $\text{CHCl}_3$ ) showed absorption at  $1740 \text{ cm}^{-1}$  indicating the presence of ester. The proton magnetic resonance spectroscopy ( $\text{CDCl}_3$ , 60 MHz) indicated the presence of six quaternary methyl groups at  $\delta$  0.72,  $\delta$  0.92, ( $2 \times \text{CH}_3$ ),  $\delta$  1.12,  $\delta$  1.38 and  $\delta$  1.50. A singlet at  $\delta$  3.62 was assigned to the ester  $-\text{OCH}_3$  protons and a multiplet at  $\delta$  5.26 (H, m,  $\text{H}-\underset{\text{the}}{\text{C}}=\text{C}$ ) indicated the presence of an olefinic proton. The mass spectrum of/methylester showed the molecular ion peak at  $m/e = 486 (\text{M}^+)$  and other peaks were found to be present at 456, 396, 351, 315, 262, 203, 133 and 81. The spectroscopic data of the diacetate and the methylester were found to correspond to those reported in the literature for hederagenin diacetate (84) and hederagenin methylester (85). This further confirmed the identity of the sapogenin II as hederagenin (83).

### iii) Isolation and Structure of Ursolic Acid

The fresh plants were extracted with distilled water. The aqueous solution was found to contain a mixture of saponins. The aqueous solution of saponins was subjected to acidic hydrolysis in order to obtain the crude

sapogenins. These were chromatographed on a silica gel column and the column eluted with petroleum-ether, petroleum ether-chloroform and chloroform-methanol. A sapogenin III was isolated from the petroleum ether-chloroform fraction (FG<sub>2</sub>) and the chloroform fraction (FG<sub>3</sub>).

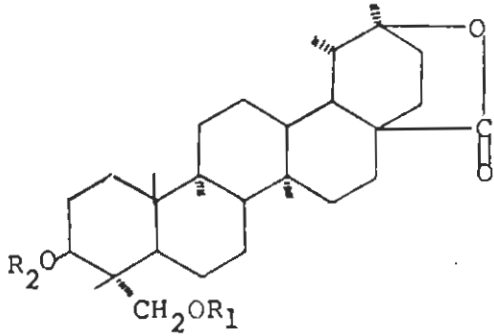
The fractions (FG<sub>2</sub>) and (FG<sub>3</sub>)<sup>on</sup>/evaporation afforded a crystalline material which was further purified by repeated crystallisation in methanol. The substance gave a positive Liebermann-Burchard test for triterpenes and a red colouration with trichloroacetic acid which indicated it to be a pentacyclic triterpene.<sup>165</sup> It melted at 292°C. An accurate mass measurement on the molecular ion afforded M<sup>+</sup> at m/e = 456.3624 in agreement with formula C<sub>30</sub>H<sub>48</sub>O<sub>3</sub>. The I.R. (KBr disc) showed an absorption at 3400 cm<sup>-1</sup> indicating the presence of -OH group while another absorption at 1700 cm<sup>-1</sup> was attributed to the presence of an acid carbonyl group. The proton magnetic resonance spectrum (CD<sub>3</sub>OD, 100 MHz) indicated the presence of seven quaternary methyl groups at  $\delta$  0.72,  $\delta$  0.80,  $\delta$  0.97 (2 x CH<sub>3</sub>),  $\delta$  1.00, (2 x CH<sub>3</sub>) and  $\delta$  1.16. A multiplet at  $\delta$  5.28 indicated the presence of an olefinic proton. The mass spectrum showed the molecular ion peak at m/e = 456 (M<sup>+</sup>) and other major peaks were at 438, 428, 110, 297, 248 and 203. On the basis of the above spectroscopic data the

sapogenin III was identified as ursolic acid (86). This was confirmed by direct spectroscopic and chromatographic comparisons with an authentic sample.

This was further confirmed by preparing the corresponding acetate with acetic anhydride and pyridine at room temperature. The substance melted at 289°C and afforded the molecular ion peak at  $m/e = 498$  ( $M^+$ ) indicating the presence of one acylable OH group. The I.R. spectrum ( $CHCl_3$ ) showed an absorption at  $1735\text{ cm}^{-1}$  indicating the presence of an ester grouping. The proton magnetic resonance spectrum ( $CDCl_3$ , 60MHz) showed the presence of seven quaternary methyl groups in the region  $\delta 0.75 - \delta 1.92$ . A singlet appeared at  $\delta 2.33$  corresponding to the methyl protons of the acetyl group and a multiplet at  $\delta 5.24$  (H, m,  $H-\underset{|}{C}=C<$ ) due to the olefinic proton. The mass spectrum of ursolic acid acetate afforded the molecular ion peak at  $m/e = 498$  and other peaks appeared at  $m/e = 248, 203, 189, 135$  and  $133$ .

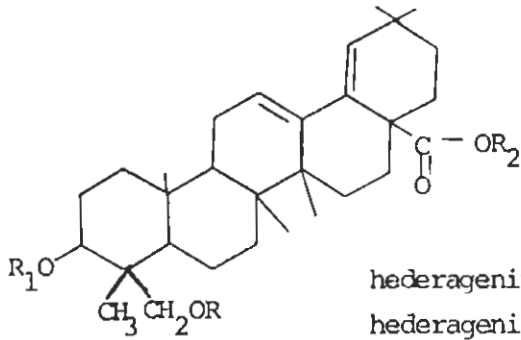
Esterification of ursolic acid with diazomethane afforded the methylester. The I.R. spectrum ( $CHCl_3$ ) showed absorption at  $1737\text{ cm}^{-1}$  indicating the presence of an ester group.

The proton magnetic resonance spectrum ( $\text{CDCl}_3$ , 60MHz) indicated the presence of seven quaternary methyl groups in the region  $\delta$  0.75 -  $\delta$  1.90. A singlet at  $\delta$  3.60 was assigned to the ester  $-\text{OCH}_3$  protons and a multiplet appeared at  $\delta$  5.26 (H, m,  $\text{H}-\underset{\text{f}}{\text{C}}=\text{C}<$ ) due to the olefinic proton. The ester showed the molecular ion peak at  $m/e = 470$  ( $\text{M}^+$ ) while other peaks were present at  $m/e = 455, 411, 410, 267, 244, 207, 203, 189$  and 133. The spectroscopic data of the acetate and the methylester were found to correspond to those reported in the literature for ursolic acid acetate (87) and ursolic acid methylester (88). This further confirmed the identity of the sapogenin III as ursolic acid (86).



nahagenin (81),  $R_1=R_2=H$

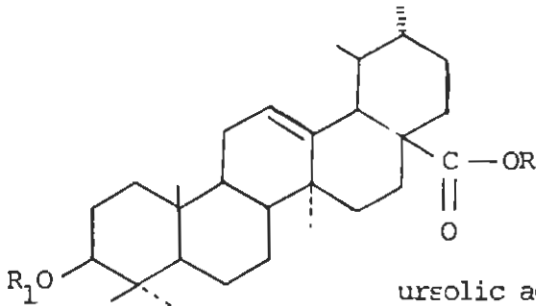
nahagenin diacetate (82),  $R_1=R_2=O=C-CH_3$



hederagenin (83),  $R_1=R_2=H$

hederagenin diacetate (84)  $R=R_1=O=C-CH_3$   
 $R_2=H$

hederagenin methylester (85)  $R_2=CH_3$   
 $R=R_1=H$



ursolic acid (86)  $R_1=R=H$

ursolic acid acetate (87)  $R_1=O=C-CH_3$

$R=H$

ursolic acid methylester (88)  $R_1=H$

$R=CH_3$

B. ISOLATION AND STRUCTURES STUDIES ON SAPONINS  
OF FAGONIA INDICA

The plant *Fagonia indica* was studied for its saponin content. The crude saponins fraction (F) was further fractionated into two fractions (FS<sub>1</sub> and FS<sub>2</sub>) by flash column chromatography, using silica gel (G 60). Elution was carried out first with CHCl<sub>3</sub> : MeOH : EtOAc (2:2.5:3.5) to afford the first fraction (FS<sub>1</sub>). This contained four different saponins as shown by t.l.c. one of which was present in major quantities and was designated as saponin "L". The second fraction (FS<sub>2</sub>) afforded two saponins, "R" and "X" along with pinacol.

i) Isolation and Structure of Saponin "L" (89)

The fraction (FS<sub>1</sub>, 2.0g) was chromatographed on a column packed with silica gel (G 60) and elution was carried out with CHCl<sub>3</sub> : MeOH : EtOAc (2:2 : 3.5). The fraction containing the partially purified saponin "L" (89) was subjected to preparative t.l.c. on silica gel plates using the solvent system CHCl<sub>3</sub> : MeOH : EtOAc (2:2.5:3.5) whereby 65mg of the pure saponin "L" (89) was obtained.

The saponin "L" (89) was refluxed with 5% HCl for 4 hours on a boiling water bath. The reaction mixture was extracted with  $\text{CHCl}_3$  to obtain the corresponding aglycone. The chloroform was evaporated to a brownish gum which was found by t.l.c. to consist mainly of the aglycone "A" which was further purified by thin layer chromatography on sheets of silica gel (G 60) in  $\text{CHCl}_3$ : MeOH (94:6). The aglycone "A" was identified as hederagenin (83) with the help of spectroscopic comparisons and particularly by its characteristic  $^{13}\text{C}$ -nuclear magnetic resonance spectrum. The olefinic carbons C-12 and C-13 resonated at  $\delta$  122.2 and  $\delta$  143.6 respectively which indicated that aglycone "A" belonged to the olean-12-en series of triterpenoids.<sup>166</sup> The other chemical shifts were identical to those reported for hederagenin.<sup>166</sup> The identity of aglycone "A" was further confirmed by direct chromatographic comparison with an authentic sample of hederagenin (83).

The sugar unit was identified as follows. The saponin "L" was taken in 1 ml of dioxane and 1ml of trifluoroacetic acid (2.0 molar solution) was added. The solution was heated at 100°C for 12 hours. The reaction mixture was evaporated to dryness, reduced with  $\text{NaBH}_4$  in water

and then treated with acetic anhydride and pyridine to afford the corresponding acetate which was subjected to gas chromatography. The retention time of the alditol acetate was found to be equal to the retention time of hexitol hexacetate on a OV-225 column at 170°C. The presence of glucose was confirmed by GC/MS whereby the ( $M^+$  OAc) peak was recorded at m/e 289 while other peaks were present at 259, 217, 187, 170, 145, 115. It has been demonstrated that molecular ion peaks of alditol acetates are not generally observed but peaks corresponding to  $M^+$  OAc or  $M^+$ -CH<sub>2</sub>OAc<sup>79,174,175</sup> have usually been recognized.

The saponin "L" (89) was refluxed with 0.1% NaOH (10ml) in dioxane (10ml) for 2 hours. The reaction mixture was extracted with n-BuOH. The n-BuOH layer on evaporation afforded prosapogenin "PL" which was purified by thin layer chromatography on sheets of silica gel (G 60) in CHCl<sub>3</sub>:MeOH:EtOAc (2:2:3.5).

It has been demonstrated that when C-1 of a glucose molecule is linked with the C-28 carbonyl group of a triterpenoid aglycone then C-1 of glucose resonates in the region  $\delta$  94--96 ppm. On the other hand if C-1

of the glucose molecule is linked with the C-3 hydroxyl group of a triterpenoid aglycone then C-1 resonates in the region  $\delta 103$ - $\delta 105$ <sup>168-172</sup>. From a comparison of the <sup>13</sup>C-nuclear magnetic resonance spectrum of hederagenin (83) with the <sup>13</sup>C-nuclear magnetic resonance spectrum of prosapogenin "PL", it was clear that C-1 of glucose molecule ( $\delta 104.94$ ) was linked to the C-3 hydroxyl group of hederagenin.<sup>170</sup> Other chemical shifts of glucose moiety were observed at  $\delta 74.86$ ,  $\delta 78.65$ ,  $\delta 71.61$ ,  $\delta 73.57$  and  $\delta 61.73$ , which were assigned to carbons C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub> and C<sub>6</sub> respectively.<sup>168</sup> The <sup>13</sup>C-nuclear magnetic resonance spectrum of prosapogenin "PL" is given in Table VI. The position of the glycosidic linkage was further confirmed by analysing the C-1 anomeric proton signals in the proton magnetic resonance spectrum which showed a doublet at  $\delta 4.6$  (1H, d, J = 7.5Hz) which indicated that the glucose unit is linked to the C-3 hydroxyl group of hederagenin (83). The coupling constant (J = 7.5Hz) was consistent with a  $\beta$ -glycosidic linkage<sup>168</sup> (in  $\alpha$ -glucosidic linkages, J = 3-4 Hz). Complex multiplets appeared in the region  $\delta 2.8$ - $\delta 4.00$  due to the glucose anomeric protons. The <sup>13</sup>C-nuclear magnetic resonance spectrum of saponin "L" supported the above results and also showed the presence of one more glucose molecule which was linked to the C-28 carbonyl group of hederagenin

through C-1 of glucose ( $\delta$ 94.85).<sup>170</sup> The <sup>13</sup>C-nuclear magnetic resonance spectrum is given in Table IV. These linkages were further confirmed by analysing the anomeric proton signals in the proton magnetic resonance spectrum of saponin "L" which showed a doublet at  $\delta$  6.2 (1H, d, J = 7Hz) indicating that the glucose unit was linked to the C-28 carbonyl group of hederagenin<sup>171</sup> while the coupling constant indicated that it was linked by a  $\beta$ -glycosidic linkage. Another doublet at  $\delta$  4.6 (1H, d, J = 7.5Hz) further confirmed the presence of glucose unit attached to the C-3 hydroxyl group of hederagenin and<sup>the</sup> coupling constant (J = 7.5Hz)<sup>168,172</sup> indicated that it was linked by a  $\beta$ -glycosidic linkage. The glucose anomeric protons resonate in the region  $\delta$  2.8 -  $\delta$  4.2. The multiplet at  $\delta$  5.28 (H, m, H-C=C<) indicated the presence of olefinic proton. On the basis of these spectral data structure (89) was assigned to saponin "L" (28-O- $\beta$ -D-glucopyranosyl 3-O- $\beta$ -D-glucopyranosyl hederagenin) which is a new saponin.

ii) Isolation and Identification of Saponin "R" (90)

Two different procedures have been adopted for the isolation of saponin "R" (90). The second fraction (FS<sub>2</sub>, 5.00g) which contained two saponins was chromatographed

on a silica gel (G 60) column. Elution was carried out with  $\text{CHCl}_3$ :MeOH:EtOAc (2:3:3.5). Concentration of the appropriate fractions afforded the partially purified saponin "R" (90). Saponin "R" was further purified by repeated preparative t.l.c. on silica gel plates using  $\text{CHCl}_3$ : MeOH: EtOAc: water (2:3:3.5:1). By this procedure 19mg of pure saponin "R" was obtained. An alternative procedure applied, which was found to be more convenient, was to isolate the saponins on Sephadex LH-20 and Biogel P-2. The crude saponin fraction (F) was first chromatographed on a Sephadex LH-20; elution was carried out with a mixture of EtOH and water (1:1). The first eluates from the column afforded a mixture of three saponins namely saponin "R", saponin "X<sub>1</sub>" and saponin "X<sub>2</sub>" as a yellow gummy mass. To remove the yellow colouration the material was passed through a silica gel (G 60) column and elution was carried out with  $\text{CHCl}_3$ : MeOH: EtOAc (2:2.5:3.5). On concentration a white mixture of saponins was obtained. Saponin "R" (90) was further purified by chromatography on a column of Biogel P-2 the column being eluted with water. The saponin "R" containing fraction was finally purified by chromatography on a Sephadex LH-20 column elution being carried out with water. The other

two saponins,  $X_1$  and  $X_2$  were purified by repeated column chromatography on a Sephadex LH-20 column using water as a solvent.

Saponin "R" was hydrolysed with 5% HCl under the same conditions as described earlier for saponin "L". The aglycone "B" thus obtained was identified as hederagenin (83) with the help of spectral comparisons as well as by direct chromatographic comparison on t.l.c. with an authentic sample of hederagenin in  $\text{CHCl}_3$ :MeOH (94:6). The sugar unit was identified in the form of its alditol acetate which was subjected to gas chromatography. The retention time of the alditol acetate was found to be equal to the retention time of hexitol hexacetate on a OV-225 column at  $170^\circ\text{C}$ . The presence of glucose was confirmed by GC/MS whereby the (M-OAc) peak was recorded at m/e 289 and other peaks were present at 259, 217, 187, 170, 145 and 115.

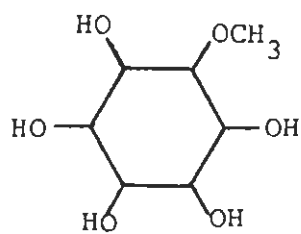
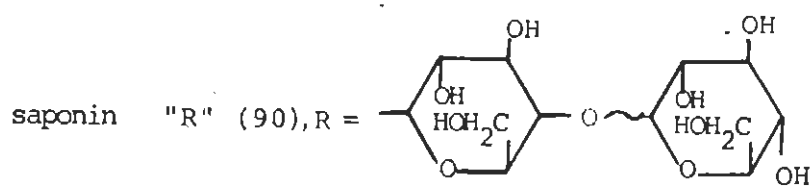
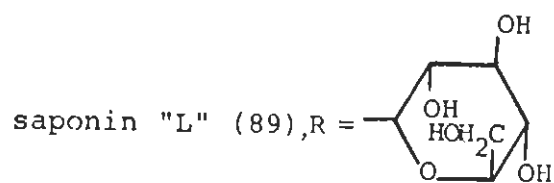
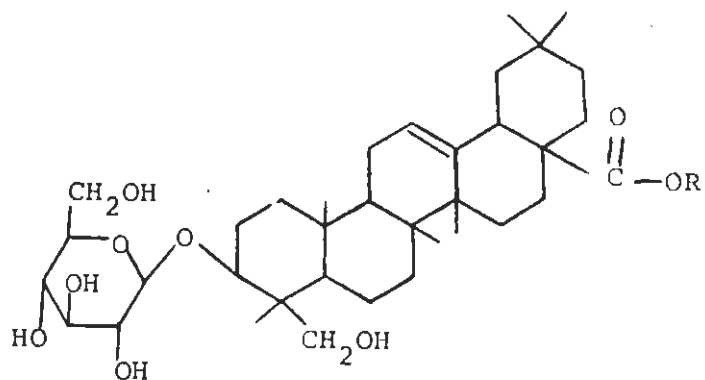
Saponin "R" was subjected to alkaline hydrolysis under the same conditions as described for saponin "L" (89). The prosapogenin "PR" thus obtained was found to be identical to prosapogenin "PL" obtained earlier by comparison of their  $^{13}\text{C}$ -nuclear magnetic resonance spectra. This was

further confirmed by their direct comparison on t.l.c. in several different solvent systems. This showed that only one glucose unit is linked to the C-3 hydroxyl group of hederagenin in saponin "R". Moreover, the  $^{13}\text{C}$ -nuclear magnetic resonance spectrum of saponin "R" indicated that C-1 of glucose molecule ( $\delta$  105.3) was linked to the C-3 hydroxyl group of hederagenin.<sup>168</sup> The  $^{13}\text{C}$ -nuclear magnetic resonance spectrum of saponin "R", also showed that the glucose molecule is linked to the C-28 carbonyl group of hederagenin through C-1 ( $\delta$  95.5) of glucose and that C-1 of another molecule of glucose ( $\delta$  103.5) was also linked to it.<sup>168</sup> Other chemical shifts of saponin "R" are given in Table-V. These linkages were confirmed by analysing the anomeric proton signals in the proton magnetic resonance spectrum. A doublet at  $\delta$  6.4 (1H, d,  $J = 7\text{Hz}$ ) indicated that the glucose molecule is linked to the C-28 carbonyl group of hederagenin and the magnitude of the coupling constant ( $J = 7\text{Hz}$ ) was indicative of the fact that a  $\beta$ -glucosidic linkage was present.<sup>168</sup> A doublet at  $\delta$  4.8 (1H, d,  $J = 7.5\text{Hz}$ ) indicated that the glucose molecule is linked to C-3 hydroxyl group of hederagenin (83) while the coupling constant ( $J = 7.5\text{ Hz}$ ) again indicated that it is linked by a  $\beta$ -glucosidic linkage.<sup>168</sup> The linkage

of the two glucose units at C-28 is under investigation. On the basis of these data saponin "R" was identified as 28-0- $\beta$ -D-glucopyranosyl $\wedge$ glucopyransoyl-3-0- $\beta$ -D-glucopyranosyl hederagenin (90) which is a new saponin.

C. ISOLATION AND IDENTIFICATION OF PINATOL FROM  
FAGONIA INDICA

The fraction (FS<sub>2</sub>) which was obtained by flash column chromatography was found to contain pinatol. The fraction was purified by preparative t.l.c. on silica gel plates (G 60) in CHCl<sub>3</sub>:MeOH:EtOAc: water (2:3:3.5:1). The pure material obtained was identified as pinatol by comparison of its <sup>13</sup>C-nuclear magnetic resonance spectrum with that reported for pinatol in the literature.<sup>173</sup> The chemical shifts of the carbon atoms of pinatol were found to occur at δ 83.0, δ 73.3, δ 72.8, δ 72.6, δ 71.7, δ 71.0 and δ 60.8 which were assigned to carbons C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, C<sub>6</sub> and OCH<sub>3</sub> respectively.



pinatol (91)

3.3

EXPERIMENTAL\*

A. Isolation and Structural Studies on Saponins of Fagonia indica

The aerial parts of the fresh plants (*Fagonia indica*) (8.0 Kg) which were collected in Karachi were finely chopped and soaked in ethyl alcohol (3 x 20 litres). The extracts were drawn regularly and after three extractions the extracts were concentrated in a cyclone evaporator under reduced pressure, the evaporation temperature being maintained between 50-55°C. A dark green coloured semi-solid residue (53.0g) was obtained which was dissolved in methanol. Addition of acetone resulted in precipitation. The precipitates were filtered, washed with petroleum ether (500ml), chloroform (500 ml), and finally with ethyl acetate (250ml). The precipitates (18.0g) were found to contain six saponins one of which was/to occur in major quantity while the others were present in minor amounts.

i) Hydrolysis of Saponins

The crude saponins (15.0g) were refluxed with 20% ethanolic HCl (300 ml) for 5 hours on a water bath. Ethanol was evaporated from the reaction mixture which was then extracted with chloroform (1.5 litre) to afford

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\* For general notes on experimental, please see page No.64.

the crude mixture of sapogenins. The chloroform layer was separated and dried (anhydrous  $\text{Na}_2\text{SO}_4$ , 30.0g) and evaporated to afford a brownish mixture of crude sapogenins (8.0g).

ii) Isolation of Nahagenin and Hederagenin

The crude mixture of sapogenins (8.0g) was chromatographed on silica gel (320.0g). The column was packed in petroleum-ether and was eluted with petroleum-ether followed by increasingly polar mixtures of petroleum ether-chloroform, chloroform and chloroform-methanol. The chloroform-methanol fraction (FG), afforded two crystalline compounds, sapogenin (I) and sapogenin (II). Sapogenin (I) was found to be a new sapogenin named as a "nahagenin" (81) while sapogenin (II) was identified as hederagenin (83). "Nahagenin" (81) was further purified by crystallisation from chloroform m.p.  $298^\circ\text{C}$ . It analysed for  $\text{C}_{30}\text{H}_{48}\text{O}_4$ : found C = 75.35%, H=10.57% calculated for  $\text{C}_{30}\text{H}_{48}\text{O}_4$  : C = 76.22% H = 10.24%.

An accurate mass measurement on the molecular ion afforded  $M^+ = 472.3540$  ( $\text{C}_{30}\text{H}_{48}\text{O}_4$  requires 472.3552) and other peaks were present at: 454.3456 ( $\text{C}_{30}\text{H}_{46}\text{O}_3$  requires 454.3446), 436.3318 ( $\text{C}_{30}\text{H}_{44}\text{O}_2$  requires 436.3341),

424.3388 ( $C_{29}H_{44}O_2$  require 424.3341), 409.3183 ( $C_{28}H_{41}O_2$  requires 409.3106), 396.3112 ( $C_{27}H_{40}O_2$  requires 396.3028), 395.2931 ( $C_{27}H_{39}O_2$  requires 395.3028).

I.R.Spectrum ( $CHCl_3$ ):  $\nu_{max}$  1740  $cm^{-1}$  (6 membered lactone C=O stretching)  
3460 (OH, stretching)

$^1H$ -N.M.R. ( $CDCl_3$ , 100 MHz):

$\delta$  0.87 (3H, s,  $\underline{CH}_3$ )  
 $\delta$  0.88 (3H, s,  $\underline{CH}_3$ ),  
 $\delta$  0.91 (3H, s,  $\underline{CH}_3$ ),  
 $\delta$  0.94 (3H, s,  $\underline{CH}_3$ ),  
 $\delta$  1.36 (3H, s,  $\underline{CH}_3$ )  
 $\delta$  1.14 (3H, s,  $\underline{CH}_3$ )

$^{13}C$ -N.M.R. ( $CDCl_3$ , 100 MHz) is given in Table-II.

Mass Spectrum: m/e = 472 ( $M^+$  5%), 454 (15%), 436 (58%), 424 (100%), 409 (38%), 395 (45%), 261 (8%).

Nahagenin Diacetate (82): Nahagenin (40 mg), acetic anhydride (0.5ml) and pyridine (0.5ml) were allowed to stand overnight at room temperature. The white solid that separated on treatment with water was extracted with

chloroform (40 ml). The chloroform layer was dried (anhydrous  $\text{Na}_2\text{SO}_4$ , 2.0g) and evaporated to a white solid (30 mg). The mass spectrum of this white solid afforded  $\text{M}^+$  at 556 ( $\text{M}^+$ ) which indicated the presence of two acylable -OH groups.

I.R.Spectrum ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  1743  $\text{cm}^{-1}$  (lactone, C=O, stretching)

$^1\text{H-N.M.R.}$  ( $\text{CDCl}_3$ , 100 MHz)

- $\delta$  0.85 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.89 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.95 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.14 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.16 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.36 (3H, s,  $\text{CH}_3$ ),
- $\delta$  2.54 (3H, s,  $\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$ )

Mass Spectrum:  $m/e = 556$  ( $\text{M}^+$ , 20%), 496 (25%), 436 (75%), 368 (20%), 323 (9%), 284 (14%), 233 (32%), 190 (100%), 161 (51%), 119 (90%).

Hederagenin(83): Hederagenin was purified by crystallisation in methanol, m.p.  $302^\circ\text{C}$  (80mg). An accurate mass measurement on the molecular ion afforded

$M^+ = 472.3525$  ( $C_{30}H_{48}O_4$  requires 472.3552).

I.R.Spectrum ( $CHCl_3$ ):  $\lambda_{max}$  3440  $cm^{-1}$  (OH, stretching)  
1700  $cm^{-1}$  (acid, C=O stretching)

$^1H$ -N.M.R. ( $CD_3OD$ , 100 MHz)

- $\delta$  0.68 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  0.70 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  0.90 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  0.93 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  0.97 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  1.16 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  5.24 (H, m,  $H-C=C<$ ).

Mass Spectrum:  $m/e = 472$  ( $M^+$ , 3%), 248 (100%), 203 (53%),  
175 (15%), 133 (14%), 119 (15%).

Hederagenin Methylene Ester (85): Hederagenin (50mg) was dissolved in methanol (3ml) treated with  $CH_2N_2$  at  $0^\circ C$  for overnight and then evaporated carefully. The evaporated mass was then taken in ether and was crystallised. The product was found to be converted to the corresponding methylester (35mg).

I.R.Spectrum ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$   $3440 \text{ cm}^{-1}$  (OH stretching)  
 $1740 \text{ cm}^{-1}$  (ester, C=O stretching).

$^1\text{H-N.M.R.}$  ( $\text{CDCl}_3$ , 60 MHz)

- $\delta$  0.72 (3H, s,  $\text{CH}_3$ )
- $\delta$  0.92 (6H, s, 2 x  $\text{CH}_3$ ),
- $\delta$  1.12 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.38 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.50 (3H, s,  $\text{CH}_3$ ),
- $\delta$  3.62 (3H, s,  $\text{OCH}_3$ ),
- $\delta$  5.26 (H, m,  $\text{H}-\underset{\text{I}}{\text{C}}=\text{C}<$ )

Mass Spectrum:  $m/e = 486 (\text{M}^+, 10\%), 456 (3\%), 396 (3\%),$   
 $351 (2\%), 315 (2\%), 262 (78\%), 203 (100\%), 133 (60\%),$   
 $81 (57\%).$

Hederagenin Diacetate (84): Hederagenin (30mg), acetic anhydride (0.5ml) and pyridine (0.5ml) were allowed to stand overnight at room temperature. The white solid that separated on treatment with water was extracted with chloroform (80 ml). The chloroform layer was dried (anhydrous  $\text{Na}_2\text{SO}_4$ , 2.0g) and evaporated to a white solid (20mg). The mass spectrum of this white solid afforded/<sup>the</sup>molecular ion peak at  $556 (\text{M}^+)$  which indicated the presence of two acylable -OH groups.

I.R.Spectrum ( $\text{CHCl}_3$ ):  $\nu_{\text{max}}$  1734  $\text{cm}^{-1}$  (ester, C=O stretching).

$^1\text{H-N.M.R.}$  ( $\text{CDCl}_3$ , 60 MHz)

- $\delta$  0.75 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.95 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.25 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.90 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.28 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.45 (3H, s,  $\text{CH}_3$ ),
- $\delta$  2.57 (3H, s,  $\text{O}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{CH}_3$ )
- $\delta$  5.24 (H, m,  $\text{H}-\underset{\text{O}}{\text{C}}=\text{C}<$ )

Mass Spectrum:  $m/e = 556$  ( $\text{M}^+$ , 1%), 496 (2%), 436 (4%), 248 (100%)  
203, (90%), 149 (34%) and 95 (52%).

iv) Isolation and Identification of Ursolic Acid

The aerial parts of the fresh plants (1.0Kg) were soaked in distilled water (2 litres). The extract was withdrawn after eight hours. This aqueous solution was found to contain a mixture of saponins and it formed strong foams on shaking. The aqueous solution of saponins was acidified by adding concentrated hydrochloric acid to pH-2 and the mixture boiled for 4 hours. The reaction mixture was then extracted with chloroform (4 litres) to obtain the crude mixture of sapogenins. The chloroform

layer was separated, dried (anhydrous  $\text{Na}_2\text{SO}_4$ , 40g) and evaporated to a brown residue. The crude mixture of sapogenins (5.0g) was chromatographed on a silica gel column (200.0g). The column was packed in petroleum-ether and the column eluted with petroleum-ether, petroleum ether-chloroform, chloroform and chloroform-methanol. Sapogenin III was isolated from the petroleum ether-chloroform and chloroform fractions. It was further purified by repeated crystallisation in methanol, m.p.  $292^\circ\text{C}$  Sapogenin III was identified as ursolic acid (86). An accurate mass measurement on the molecular ion afforded  $M^+ = 456.3624$  ( $\text{C}_{30}\text{H}_{48}\text{O}_3$  requires 456.3603).

I.R. Spectrum (KBr disc):  $\nu_{\text{max}}$   $3440 \text{ cm}^{-1}$  (OH, stretching)  
 $1700 \text{ cm}^{-1}$  (acid, C=O stretching)

$^1\text{H-N.M.R.}$  ( $\text{CD}_3\text{OD}$ , 60 MHz)

- $\delta$  0.72 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.80 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.97 (6H, s, 2 x  $\text{CH}_3$ ),
- $\delta$  1.00 (6H, s, 2 x  $\text{CH}_3$ ),
- $\delta$  1.16 (3H, s,  $\text{CH}_3$ ),
- $\delta$  5.28 (H, m,  $\text{H}-\text{C}=\text{C}<$ )

Mass Spectrum:  $m/e = 456 (M^+, 20\%), 441 (5\%), 438 (10\%), 410 (6\%), 297 (10\%), 248 (90\%), 203 (100\%), 189 (79\%), 133 (80\%).$

Ursolic Acid Methylester (88): Ursolic acid (40 mg) was dissolved in methanol (3ml). treated with  $CH_2N_2$  at  $0^\circ C$  overnight and then evaporated carefully. The evaporated mass was then taken up in ether and crystallised. The product was found to be converted to the corresponding methylester (30 mg), m.p.  $100^\circ C$ .

I.R.Spectrum ( $CHCl_3$ ):  $\nu_{max} 3440 \text{ cm}^{-1}$  (OH, stretching),  
 $1737 \text{ cm}^{-1}$  (ester, C=O stretching)

$^1H$ -N.M.R. ( $CDCl_3$ , 60 MHz)

$\delta 0.75$  (3H, s,  $\underline{CH_3}$ ),  
 $\delta 0.82$  (3H, s,  $\underline{CH_3}$ ),  
 $\delta 0.98$  (6H, s,  $2 \times \underline{CH_3}$ ),  
 $\delta 1.14$  (6H, s,  $2 \times \underline{CH_3}$ ),  
 $\delta 1.90$  (3H, s,  $\underline{CH_3}$ ),  
 $\delta 3.60$  (3H, s,  $O\underline{CH_3}$ ),  
 $\delta 5.26$  (H, m,  $H-\underset{|}{C}=C<$ )

Mass Spectrum:  $m/e = 470 (M^+, 5\%), 455 (10\%), 411 (8\%), 410 (12\%), 267 (50\%), 214 (20\%), 207 (60\%), 203 (100\%), 189 (18\%), 133 (30\%).$

Ursolic Acid Acetate (87): Ursolic acid (35mg), acetic anhydride (0.5ml) and pyridine (0.5ml) were allowed to stand for 12 hours at room temperature. The white solid that separated on treatment with water was extracted with chloroform (80ml). The chloroform layer was dried (anhydrous  $Na_2SO_4$  2.0g) and evaporated to a white solid (20mg). The mass spectrum of this white solid afforded the molecular ion at  $m/e = 498 (M^+)$ , addition of 42 mass units indicating the presence of one acylable OH group.

I.R.Spectrum (KBr disc):  $\nu_{max} 1735 \text{ cm}^{-1}$  (C=O stretching).

$^1H$ -N.M.R. ( $CDCl_3$ , 60 MHz)

- $\delta$  0.75 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  0.87 (6H, s, 2 x  $\underline{CH}_3$ ),
- $\delta$  0.94 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  1.14 (6H, s, 2 x  $\underline{CH}_3$ ),
- $\delta$  1.92 (3H, s,  $\underline{CH}_3$ ),
- $\delta$  2.33 (3H, s,  $\overset{O}{\parallel} \text{C}-\underline{CH}_3$ ),
- $\delta$  5.24 (H, m,  $\text{H}-\underset{|}{\text{C}}=\text{C} <$ ).

Mass Spectrum:  $m/e = 498 (M^+, 1\%), 438 (3\%), 248 (100\%),$   
 $203 (62\%), 189 (48\%), 135 (15\%) \& 133 (64\%).$

B. ISOLATION AND STRUCTURE STUDIES ON SAPONINS OF  
FAGONIA INDICA

The crude saponin fraction (10.0g) was fractionated by flash column chromatography on a silica gel column (360.0g). Elution was carried out with  $\text{CHCl}_3$ : MeOH:EtOAc (2:2.5:3.5) (4 litres) and  $\text{CHCl}_3$ : MeOH:EtOAc: water (2:2.5:3.5) (3 litre). The first fraction ( $\text{FS}_1$ ) was obtained on elution with  $\text{CHCl}_3$ :MeOH: EtOAc (2:2.5:3.5)(4 litre). Evaporation of the solvent fraction afforded a mixture of four saponins (2.15g), one of which was present in major quantities and was designated as saponin "L". The second fraction ( $\text{FS}_2$ ) was obtained on elution with  $\text{CHCl}_3$ :MeOH: EtOAc: water (2:3:3.5:1) (3 litre). On evaporation of the solvent the fraction afforded a mixture of two saponins namely saponin "R" and saponin "X" along with pinatol (5.72g).

i) Isolation of Saponin "L"(89)

The first fraction ( $\text{FS}_1$ , 2.0g) was chromatographed on a silica gel column (140.0g). The column was packed in  $\text{CHCl}_3$ :MeOH: EtOAc (2:2:3.5). Elution was carried out with  $\text{CHCl}_3$ : MeOH:EtOAc (2:2:3.5) (2 litres). The first eluates afforded the partially pure saponin "L"

(500 mg). The saponin "L" was further purified by preparative t.l.c. in  $\text{CHCl}_3$ :MeOH:EtOAc (2:2.5:3.5) on silica gel (2 m.m.) plates. Each plate was loaded with 60mg of the partially pure saponin "L". The pure saponin "L" (65mg) was thus obtained from six preparative t.l.c. plates.

$^1\text{H-N.M.R.}(\text{C}_5\text{D}_6\text{N}, 100\text{MHz FT})$

- $\delta$  0.90 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.93 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.97 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.65 (3H, s,  $\text{CH}_3$ ),
- $\delta$  0.69 (3H, s,  $\text{CH}_3$ ),
- $\delta$  1.16 (3H, s,  $\text{CH}_3$ ),
- $\delta$  4.6 (1H, d,  $J=7.5\text{Hz}$ , anomeric H),
- $\delta$  5.28 (1H, m,  $\text{H}-\text{C}=\text{C}$ ),
- $\delta$  6.2 (1H, d,  $J=7.0\text{Hz}$ , anomeric H)

$^{13}\text{C-N.M.R.}(\text{C}_5\text{D}_5\text{N}, 100\text{MHz FT})$  is given in Table-IV.

ii) Acid Hydrolysis of Saponin "L"

Saponin "L" (20mg) was refluxed with 5% HCl (10ml) for 4 hours on a boiling water bath. The reaction mixture was extracted with  $\text{CHCl}_3$  (15 ml x 2) to obtain the corresponding aglycone "A". The chloroform layer was separated, dried (anhydrous  $\text{Na}_2\text{SO}_4$ , 10.0g) and

evaporated to a brownish gum. The corresponding aglycone "A" was purified on thin layer chromatography sheets (silica gel, G 60) in  $\text{CHCl}_3$ : MeOH (94:6) and was identified as hederagenin with the help of spectral comparison and direct chromatographic comparison with an authentic sample of hederagenin (83) on t.l.c. in  $\text{CHCl}_3$ : MeOH (94:6).

$^{13}\text{C}$ -N.M.R. ( $\text{C}_5\text{D}_5\text{N}$ , 25 MHz, FT) is given in Table-III.

iii) Alkaline Hydrolyses of Saponin "L"

Saponin "L" (20mg) was refluxed with 0.4% NaOH (10ml) in dioxane (10ml) for 2 hours on a boiling water bath. The reaction mixture was extracted with n-BuOH (20ml) which was already saturated with water. On evaporation of the solvent prosapogenin "PL" was obtained which was purified on thin layer chromatography sheets (silica gel, G 60) in  $\text{CHCl}_3$ :MeOH: EtOAc (2:2:3.5) to afford the pure material (12mg).

$^{13}\text{C}$ -N.M.R. ( $\text{C}_5\text{D}_5\text{N}$ , 25 Hz FT) is given in Table-VI.

iv) Identification of Sugar Units in Saponin "L"

The saponin "L" (4mg) was taken up in dioxane (1ml) and trifluoroacetic acid (1ml of a 2 molar solution) was added. The solution was heated at 100°C for 12 hours, the reaction mixture was evaporated to dryness, dissolved in water (2ml) and NaBH<sub>4</sub> (10mg) added with stirring. The solution was allowed to stand for 2 hours. Then Dowex 50 (H<sup>+</sup>) was added to the reaction mixture till pH-3 was attained. The solution was filtered, evaporated to dryness by repeated addition of methanol (3x2ml) and treated with acetic anhydride (0.5ml) and pyridine (0.5ml). The solution was heated for 20 minutes on a boiling water bath. It was then evaporated to dryness by repeated addition of ethanol (3x2ml) and evaporation to afford the corresponding acetate (2mg). The acetate was subjected to gas chromatography. The retention time of the alditol acetate was found to be equal to the retention time of hexitol hexaacetate on OV-225 column at 170°C.

Mass Spectrum: m/e = 298 (M<sup>+</sup>-OAc, 15%), 259 (15%), 217 (30%), 187 (40%), 170 (35%), 145 (56%), 115 (100%) and 103 (50%).

v) Isolation of Saponin "R" (90)

The second fraction (FS<sub>2</sub>, 5.0g) was chromatographed on a silica gel column (175.0g). The column was packed in CHCl<sub>3</sub>: MeOH: EtOAc (2:3:3.5). Elution was carried out with CHCl<sub>3</sub>: MeOH: EtOAc (2:3:3.5) (3 litre). The partially pure saponin "R" (380 mg) was further purified by repeated preparative t.l.c. on silica gel (G 60) plates (2 m.m.) in CHCl<sub>3</sub>: MeOH: EtOAc: water (2:3:3.5:1). Each plate was loaded with 60mg of the crude saponin "R". The pure saponin "R" was thus obtained from this procedure (19mg).

vi) Second Procedure for Isolation of Saponin "R"

The crude saponin fraction (5.0g) was column chromatographed on Sephadex LH-20. The column was packed in EtOH: water (1:1) and elution was carried out with the same solvent (5 litre). Evaporation of the first eluates obtained from the column afforded a light yellow coloured mixture of three saponins, namely saponin R, saponin X<sub>1</sub> and saponin X<sub>2</sub>. This mixture was passed through a column of silica gel (G 60, 70.0g) to remove the yellow colour. The column was packed in CHCl<sub>3</sub>: MeOH: EtOAc (2:2.5:3.5). Elution was carried out with CHCl<sub>3</sub>: MeOH: EtOAc (2:2.5:3.5)

(1.5 litre). On concentration of the solvent eluates a white mixture of saponins (1.8g) was obtained. Saponin "R" was further purified on a column of Biogel-P<sub>2</sub>. The column was packed in water and elution was also carried out with water (1.5 litre). The final purification of saponin "R" was carried out by a second column chromatography on Sephadex LH-20. The column was again packed in water and elution was carried out with water (1.5 litres). A new saponin "R" (25mg) having the structure (90) was purified by this procedure. Two other saponins namely saponin X<sub>1</sub> and saponin X<sub>2</sub> were purified by repeated column chromatography on Sephadex LH-20 using water as a solvent. Structures of saponin X<sub>1</sub> and saponin X<sub>2</sub> are under investigation. All these saponins are being reisolated in larger quantities to record the other spectral data for further confirmation of their structures.

<sup>1</sup>H-N.M.R. (C<sub>5</sub>D<sub>5</sub>N, 100MH FT)

δ 0.65 (3H, s, CH<sub>3</sub>),

δ 0.70 (3H, s, CH<sub>3</sub>),

δ 0.85 (3H, s, CH<sub>3</sub>),

δ 0.93 (3H, s, CH<sub>3</sub>),

δ 0.98 (3H, s, CH<sub>3</sub>),

δ 1.16 (3H, s, CH<sub>3</sub>);

$\delta$  4.8 (1H, d,  $J=7.5\text{Hz}$ , anomeric H),

$\delta$  5.20 (1H, m, H  $\rightarrow\text{C}=\text{C}$  <),

$\delta$  6.4 (1H, d,  $J=7.00\text{ Hz}$ , anomeric H).

$^{13}\text{C}$ -N.M.R ( $\text{C}_5\text{D}_5\text{N}$ , 25MHz, FT) is given in Table-V.

vii) Acid Hydrolysis of Saponin "R"

Saponin "R" (10mg) was refluxed with 5% HCl (5ml) for 4 hours on a boiling water bath. The reaction mixture was extracted with  $\text{CHCl}_3$  (10 ml x 2) to obtain the corresponding aglycone "B". The chloroform layer was separated, dried (anhydrous  $\text{Na}_2\text{SO}_4$ , 10.0g) and evaporated to a brownish gum. The aglycone "B" (10mg) was purified on thin layer chromatography sheets (silica-gel, G 60) in  $\text{CHCl}_3$ : MeOH (94:6). The aglycone "B" was identified as hederagenin by chromatographic and spectral comparisons.

viii) Alkaline Hydrolysis of Saponin "R"

The saponin "R" (20mg) was refluxed with 0.4% NaOH (10ml) in dioxane (10ml) for 2 hours on a boiling water bath. The reaction mixture was extracted with n-BuOH saturated with water. On concentration of the solvent the crude prosapogenin "PR" was obtained which was purified by thin layer chromatography on silica gel (G 60) sheets in

CHCl<sub>3</sub>: MeOH: EtOAc (2:2:3.5) to obtain the pure prosapogenin "PR" (12mg).

<sup>13</sup>C-N.M.R. (C<sub>5</sub>D<sub>5</sub>N, 25 MHz FT) is given in Table-VII.

ix) Identification of Sugar Units in Saponin 'R'

The saponin "R" (4mg) was taken up in dioxane (1ml) and trifluoroacetic acid (1ml of a 2 molar solution) was added. The solution was heated at 100°C for 12 hours, the reaction mixture was evaporated to dryness, dissolved in water (2ml) and NaBH<sub>4</sub> (10mg) added with stirring. The solution was allowed to stand for 2 hours. Then Dowex 50(H)<sup>+</sup> was added to the reaction mixture till pH-3 was obtained. The solution was filtered, evaporated to dryness by repeated addition of methanol (3x2ml) and treated with acetic anhydride (0.5ml) and pyridine (0.5ml). The solution was heated for 20 minutes on a boiling water bath. It was then evaporated to dryness by repeated addition of ethanol (3x2ml) and evaporation to afford the corresponding acetate (2mg). The acetate was subjected to gas chromatography. The retention time of the alditol acetate was found to be equal to the retention time of hexitol hexacetate on OV-225 column at 170°C.

Mass Spectrum:  $m/e = 298$  ( $M^+ - OAc$ , 15%), 259 (15%),  
217 (30%), 187 (40%), 170 (35%), 145 (56%), 115 (100%),  
103 (50%).

ISOLATION OF PINATOL FROM FAGONIA INDICA

The fraction (FS<sub>2</sub>, 2.0g) was subjected to preparative t.l.c. on silica gel plates (2mm) in CHCl<sub>3</sub>: MeOH: EtOAc: water (2:3:3.5 1). Each plate was loaded with fraction FS<sub>2</sub> (100mg) and pinatol (25mg) was obtained from 20 preparative plates.

<sup>13</sup>C-N.M.R. (C<sub>5</sub>D<sub>5</sub>N, 25 MHz, FT)

$$C_1 = \delta 83.0$$

$$C_2 = \delta 73.3$$

$$C_3 = \delta 72.8$$

$$C_4 = \delta 72.6$$

$$C_5 = \delta 71.7$$

$$C_6 = \delta 71.0$$

$$OCH_3 = \delta 60.8$$

T A B L E - I I

<sup>13</sup>C-N.M.R. CHEMICAL SHIFT VALUES OF NAHAGENIN

Carbon	Chemical Shift(s) (ppm)	Multiplicity	Carbon	Chemical Shift(s) (ppm)	Multiplicity
C(1)	38.51	d.d	C(16)	25.15	t
C(2)	27.28	t	C(17)	42.06	s
C(3)	76.54	d	C(18)	50.51	d
C(4)	41.86	s	C(19)	48.37	d
C(5)	49.99	d	C(20)	84.27	s
C(6)	18.35	t	C(21)	41.98	t
C(7)	32.22	t	C(22)	33.68	t
C(8)	40.51	s	C(23)	71.92	d.d
C(9)	42.83	d	C(24)	11.30	q
C(10)	37.05	s	C(25)	15.69	q
C(11)	20.96	t	C(26)	16.63	q
C(12)	27.05	t	C(27)	23.98	q
C(13)	27.59	d	C(28)	177.29	s
C(14)	41.08	s	C(29)	14.26	q
C(15)	26.87	t	C(30)	23.98	q

T A B L E - III

<sup>13</sup>C-N.M.R. CHEMICAL SHIFT VALUES OF HEDERAGENIN

Carbon	Chemical Shift(δ) (ppm)	Carbon	Chemical Shift(δ) (ppm)
C(1)	38.1	C(16)	23.4
C(2)	26.4	C(17)	46.7
C(3)	76.4	C(18)	41.3
C(4)	41.7	C(19)	45.9
C(5)	49.7	C(20)	30.7
C(6)	18.5	C(21)	33.9
C(7)	32.4	C(22)	32.4
C(8)	39.3	C(23)	71.3
C(9)	47.5	C(24)	11.6
C(10)	36.9	C(25)	15.7
C(11)	23.1	C(26)	16.9
C(12)	122.2	C(27)	26.0
C(13)	143.6	C(28)	177.9
C(14)	41.7	C(29)	33.1
C(15)	27.7	C(30)	23.6

T A B L E - I V

<sup>13</sup>C-N.M.R. CHEMICAL SHIFT VALUES OF SAPONIN "L"

Carbon	Chemical Shift(s) (ppm)	Carbon	Chemical Shift(s) (ppm)
C(1)	38.5	C(16)	23.2
C(2)	26.2	C(17)	47.2
C(3)	82.3	C(18)	41.5
C(4)	42.4	C(19)	46.1
C(5)	48.5	C(20)	30.5
C(6)	18.6	C(21)	33.7
C(7)	32.4	C(22)	32.5
C(8)	39.9	C(23)	69.1
C(9)	47.8	C(24)	13.5
C(10)	37.1	C(25)	15.9
C(11)	23.4	C(26)	17.2
C(12)	122.4	C(27)	26.0
C(13)	143.8	C(28)	174.45
C(14)	41.9	C(29)	32.0
C(15)	28.1	C(30)	23.8
Glc C(1)	105.2		
Glc C'(1)	94.85		

T A B L E - V

<sup>13</sup>C-N.M.R. CHEMICAL SHIFT VALUES OF SAPONIN "R"

Carbon	Chemical Shift(s)(ppm)	Carbon	Chemical Shift(s)(ppm)
C(1)	38.7	C(16)	23.0
C(2)	26.0	C(17)	47.1
C(3)	82.4	C(18)	41.6
C(4)	43.0	C(19)	46.4
C(5)	47.8	C(20)	30.6
C(6)	18.5	C(21)	34.0
C(7)	33.0	C(22)	32.8
C(8)	39.5	C(23)	68.9
C(9)	47.6	C(24)	13.7
C(10)	37.0	C(25)	16.1
C(11)	23.5	C(26)	17.4
C(12)	122.4	C(27)	26.1
C(13)	143.7	C(28)	174.35
C(14)	42.0	C(29)	33.0
C(15)	28.5	C(30)	23.6
Glc C(1)	105.3		
Glc C(1)	95.5		
Glc C(1)	103.5		

T A B L E - V I

<sup>13</sup>C-N.M.R. CHEMICAL SHIFT VALUES OF PROSAPOGENIN "PL"

Carbon	Chemical Shift(s) (ppm)	Carbon	Chemical Shift(s) (ppm)
C(1)	38.7	C 16	23.5
C(2)	26.4	C 17	47.1
C(3)	82.0	C 18	41.5
C(4)	43.1	C 19	46.2
C(5)	47.8	C 20	30.5
C(6)	18.4	C 21	34.0
C(7)	33.1	C 22	32.8
C(8)	39.3	C 23	68.5
C(9)	47.5	C 24	13.5
C(10)	37.0	C 25	16.0
C(11)	23.5	C 26	17.3
C(12)	122.4	C 27	26.1
C(13)	143.5	C 28	176.3
C(14)	42.1	C 29	33.0
C(15)	28.4	C 30	23.6
Glc C(1)	104.94		
C(2)	74.86		
C(3)	78.65		
C(4)	71.61		
C(5)	73.57		
C(6)	61.73		