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Saponins from the roots of *Mimosa hamata* Willd.

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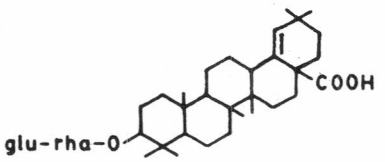
A new triterpenic saponin (saponin A) has been isolated from the roots of *Mimosa hamata* Willd. Acidic hydrolysis of saponin A yields glucose and rhamnose with morolic acid as the genin moiety. On the basis of acidic, basic and Kiliani hydrolysis and IR, FAB MS and ^{13}C NMR studies, its partial structure has been assigned as 3-O-D-glucosyl-(1→2)-L-rhamnosylmorolic acid **1** (saponin A). Alongwith this new saponin, three known saponins (mimonoside A, B and C) have also been isolated.

A survey of literature revealed the isolation of triterpenic saponins from some species of *Mimosa* genus[1-5], but only one reference on *Mimosa hamata* reporting the isolation of 4-ethylgallic acid is available[6], hence the investigation of saponins of this plant was undertaken.

During the course of our studies on the saponins of the roots of *M. hamata* we have isolated a new triterpenic saponin designated as saponin A. Partial structure **1** has been assigned on the basis of acidic, basic and Kiliani hydrolysis and spectral studies. Three known saponins (mimonoside A, B and C) were also isolated along with the new saponin.



(1)

The roots of *Mimosa hamata* Willd. were collected from Jaipur-Ajmer Road near Ajmer. The methanolic extract of the roots was suspended in water and fractionated with pet. ether. During extraction with pet. ether three layers separated out. The upper pet. ether layer, the lower aqueous layer and in between was the middle layer which appeared to be a sort of emulsion. The emulsion layer and lower aqueous layer gave positive test for saponins (blue-violet colour with vanillin-sulphuric acid spray on TLC). The lower aqueous layer was further treated with *n*-butanol and acetone to afford a crude saponin mixture. On CC the upper pet. ether layer gave β-sitosterol, emulsion (saponin rich) crude gave β-sitosterol and the new saponin **1** and lower aqueous layer gave three saponins, mimonoside A, B and C.

Saponin A (3-O-D-glucosyl-(1→2)-L-rhaosylmorolic acid) was purified by repeated CC over silica gel as white amorphous powder. It showed homogeneous behaviour on TLC, m.p. 220-25°C (dec.).

In the IR spectrum of **1** peaks appeared at 3500-2580 (broad, O-H stretching), 1740 (C=O stretching) and 1070 cm^{-1} (C-O stretching) indicating the presence of carboxylic and alcoholic groups. A peak appeared at 1640 cm^{-1} due to C=C stretching.

Acid hydrolysis of saponin A yielded the genin, m.p. 270-71° which gave positive Liebermann Burchard test and TNM test. It was identified as morolic acid[7,8] (IR and MS) and formed an acetate, m.p. 154-56° (ref. 9). The soluble hydrolysate was neutralized with $BaCO_3$. This concentrated hydrolysate was found to contain two sugars on co-paper chromatography which were identified as D-glucose (R_f 0.12) and L-rhamnose (R_f 0.32)[10]. The presence of two sugars was also indicated in ^{13}C NMR spectrum where two anomeric signals appeared at δ 103.4 and 100.4[3].

The sequence of sugars in sugar chain was identified by Kiliani hydrolysis. On hydrolysis D-glucose emerged out first (on PC) and L-rhamnose came out later, so D-glucose must be the terminal sugar. With passage of time the intensity of the spots of sugars on PC remained as such, indicating the ratio of sugars to be 1:1. This sequence of

sugars in sugar chain was further supported by FAB mass spectrum. The important peaks appeared at m/z 765 $(M+H)^+$, 603 $[(M+H)-162, glu)^+$ and 457 $[(M+H)-(162, glu+146, rha)]^+$.

The attachment of sugar chain to genin moiety was possible at two positions, the $-OH$ group at C-3 or the $-COOH$ group at C-28. The appearance of a broad peak in IR spectrum at 3500-2580 cm^{-1} due to $-COOH$ group indicated the sugar linkage to be at C-3. This was further confirmed by the basic hydrolysis of **1**, which was recovered unchanged indicating the absence of an ester linkage.

Permethylation by modified Hakomori's method[11] followed by methanolysis and hydrolysis furnished 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-L-rhamnose which confirmed that D-glucose was the terminal sugar linked with L-rhamnose at position 2.

Thus the partial structure of saponin A (3-*O*-D-glucosyl-(1→2)-L-rhamnosylmorolic acid) can be reported as **1**.

Mimonoside A was obtained as a white powder, m.p. 248-50° (dec.). On acid hydrolysis it gave an aglycone, m.p. 305°C which was identified as oleanolic acid[12]. The aqueous hydrolysate was found to contain L-arabinose, D-xylose, D-glucose and L-rhamnose on PC. Basic hydrolysis of mimonoside A afforded a prosapogenol, m.p. 240-41°C and a sugar moiety which was identified as L-rhamnose (on PC)[10] which was attached to the genin moiety by ester linkage. The number and ratio of sugars were confirmed by Kiliani hydrolysis of prosapogenol and ^{13}C NMR spectrum (seven anomeric carbons appeared at δ 95.1, 99.8, 106.2, 105.4, 102.9, 105.6 and 102.6). All spectral data (IR, ^{13}C NMR and FAB mass) closely resembled those of mimonoside A (3-*O*-{[(α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl-(1→3))-(α-L-arabinopyranosyl-(1→4))-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosyloleanolic acid) reported earlier from the bark of *Mimosa tenuiflora*[2].

Mimonoside B was obtained as a light buff amorphous powder, m.p. 240-42°. On acid hydrolysis this compound also gave oleanolic acid[12] as genin, m.p. 305° and neutralized aqueous hydrolysate indicated the presence of L-rhamnose, D-xylose, D-glucose and L-arabinose. On basic hydrolysis, the compound (mimonoside B) remained unchanged indicating the absence of ester linkage. The number and sequence of sugars in sugar chain was confirmed by ^{13}C NMR spectrum (six anomeric carbons were observed at δ 99.5, 106.4, 105.0, 102.7, 105.2 and 102.4) and FAB MS respectively. The IR, ^{13}C NMR and mass spectra were in close agreement with those of mimonoside B (3-O-{[(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3))-(α-L-arabinopyranosyl-(1→4))-β-D-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl}oleanolic acid)[2].

Mimonoside C was obtained as a white amorphous powder, m.p. 252-54°. Acid hydrolysis gave an aglycone, m.p. 298-301°, identified as the triterpene, machaerinic acid[13] and the neutralized aqueous hydroxylate indicated the presence of L-arabinose, L-rhamnose, D-xylose and D-glucose on PC. Seven anomeric peaks appeared in the ^{13}C NMR spectrum (δ 95.4, 99.9, 106.4, 105.7, 102.4, 105.5 and 103.0) indicating the number of sugar moieties. In this compound one sugar moiety(L-rhamnose) is attached to genin by ester linkage (basic hydrolysis). On the basis of comparative spectral data, the compound was identified as mimonoside C(3-*O*-{[(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3))-(α-L-arabinopyranosyl-(1→4)-βD-xylopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4))] - β-D-glucopyranosyl}-28-*O*-α-L-rhamnopyranosylmachaerinic acid) reported earlier from this genus[3].

Experimental Section

General. IR spectra were recorded (as KBr pellets) on a Perkin-Elmer model 557 spectrophotometer. ^{13}C NMR spectra were recorded on model Jeol FX 90Q at 22.49 MHz using DMSO-d_6 as solvent and TMS as internal standard. FAB MS were recorded on a Jeol SX 120/DA-6000 mass spectrometer/Data system using Argon (6 KV, 10 mA) as a FAB gas [accelerating voltage - 10 KV and matrix *m*-nitrobenzyl alcohol (NBA)].

Column chromatography was carried out using silica gel G. Paper chromatography was carried out on Whatmann No. 1 paper sheets using the solvent system *n*-BuOH-AcOH-H_2O (4:1:5, upper layer) and spots were visualized with aniline hydrogen phthalate. TLC of saponins was carried out using $CHCl_3$-MeOH-H_2O (5:4:1) as irrigant and sprayed with vanillin-sulphuric acid.

Extraction. The roots were air-dried, powdered and exhaustively extracted with methanol. The concentrated methanolic extract was suspended in water and fractionated with pet. ether, when three layers were formed, upper pet. ether layer, lower

aqueous layer and in between was the middle layer which appeared to be a sort of emulsion.

Upper layer (pet. ether fraction). Column chromatography of this fraction afforded β-sitosterol only.

Middle layer. The concentrated extract had frothing tendency on agitation and tested positive for saponins. This was chromatographed over silica gel column, elution being carried out with $CHCl_3$ followed by chloroform-methanol mixtures in order of increasing polarity when elution with $CHCl_3$ afforded β-sitosterol and $CHCl_3$:MeOH (4:1) afforded saponin A.

Saponin A. The reddish-brown solid obtained on evaporation was dissolved in a minimum amount of methanol and precipitated with chloroform as a creamy coloured powder. As it did not give a single spot, the process of chromatography and precipitation was repeated several time when a white amorphous powder, m.p. 220-25°C (dec.), was obtained; IR (KBr, cm^{-1}); 3500-2580 (broad), 2930, 2860, 1740, 1640, 1480, 1380, 1365, 1250, 1070, 1030, 890 and 810; ^{13}C NMR (DMSO-d_6): δ 182.8 (COOH), 136.5, 132.9, 103.4, 100.4, 84.9, 75.3, 73.4, 73.2, 72.5, 71.6, 70.0, 69.2, 61.4, 55.7, 51.4, 48.3, 42.5, 41.0, 40.5, 39.1, 38.7, 37.3, 34.5, 33.5, 33.5, 33.4, 32.0, 30.5, 29.5, 29.4, 28.2, 27.5, 26.1, 21.2, 18.3, 18.2, 16.5, 16.0, 15.5 and 15.0; FAB MS: m/z 765 $(M+H)^+$, 603, 457, 440, 439, 396, 249, 208, 207, 206, 205, 193, 192, 191, 190, 188, 178, 176, 164, 163, 142, 141, 95, etc.

Isolation of genin. Acid hydrolysis of saponin A was carried out with 10% H_2SO_4 by refluxing for 4 hr on a steam-bath[14]. The reaction mixture was cooled and then extracted with ether. The ether layer on removal of solvent gave the aglycone, which was crystallized from methanol as white needles, m.p. 270-71°; acetate: m.p. 254-56° (ref. 9). It was identified as morolic acid by direct comparison (m.m.p., TLC and IR) with an authentic sample.

Isolation of sugars. The aqueous hydrolysate obtained by the acid hydrolysis was neutralized with freshly prepared $BaCO_3$, filtered, concentrated and subjected to PC. The sugar portion gave two spots corresponding to D-glucose (R_f 0.12) and L-rhamnose (R_f 0.32)[10].

Alkaline hydrolysis of saponin A. The saponin A was hydrolysed with 5% KOH under reflux for 3 hr. The reaction mixture was extracted with *n*-BuOH. The aqueous layer was neutralized with 1 *N* HCl and subjected to paper chromatography which showed the absence of any spot corresponding to sugar moiety, and BuOH layer afforded saponin A as such, showing the absence of ester linkage.

Kiliani hydrolysis. Saponin A was kept with Kiliani mixture (10 mL, AcOH-H_2O-35% HCl; 35:55:10) at room temperature[15]. The reaction mixture was examined by PC. After 5 hr PC showed a spot due to D-glucose. One more spot corresponding to that of L-rhamnose appeared after 24 hr. After36 hr and 48 hr no change was observed on PC indicating the absence of any other sugar moiety.

Lower aqueous layer. The lower aqueous layer was treated with *n*-butanol and this butanol layer was then precipitated with acetone. Column chromatography of this residue on elution with $CHCl_3$ and $CHCl_3$-MeOH mixtures afforded mimonoside A, B and C.

Mimonoside A and Mimonoside B. Fractions eluted with $CHCl_3$-MeOH (7:3) gave two spots on TLC. It was dissolved in MeOH and precipitated with $CHCl_3$ as cream coloured solid which was rechromatographed over a column of silica gel. Elution with $CHCl_3$-MeOH (7:3) afforded mimonoside A followed by mimonoside B.

Mimonoside A. It was finally purified by reprecipitation to afford a white powder, m.p. 248-50°C (dec.); IR (KBr, cm^{-1}): 3300-2850 (broad), 1720, 1645, 1470, 1385, 1360, 1075, 900 and 840; FAB MS: m/z 1469 $(M+H)^+$, 1323, $[(M+H)-146]^+$, 1191 $[(M+H)-(146+132)]^+$, 1177 $[(M+H)-(2\times146)]^+$, 1059 $[(M+H)-(146+2\times132)]^+$, 1045$[(M+H)-(2\times146+132)]^+$, 1015 $[(M+H)-(2\times146+162)]^+$, 913 $[(M+H)-(2\times146+2\times132)]^+$, 751 $[(M+H)-(2\times146+2\times132+162)^+$, 619 $[(M+H)-(2\times146+3\times132+162)]^+$, 457 $[(M+H)-(2\times146+3+132+2\times162)]^+$, 438, 249, 236, 205, 204, 190, 163, 161, 148, 135, 134, 120, 99... etc. Acid hydrolysis afforded white needles, m.p. 305° (acetate: m.p. 268-69°)[12], identified as oleanolic acid. The neutralized aqueous hydrolysate on PC showed the presence of D-glucose (R_f 0.12), L-arabinose (R_f 0.18), D-xylose (R_f 0.20) and L-rhamnose (R_f 0.32).

On basic hydrolysis, the PC of aqueous layer showed one spot corresponding to L-rhamnose and the dried butanol layer gave a prosapogenol, m.p. 240-41°. This prosapogenol was treated with Kiliani mixture[15]. On PC after 10 hr, it showed three spots corresponding to L-rhamnose, L-arabinose and D-xylose. PC after 24 hr showed one more spot corresponding to D-glucose and intensity of D-xylose increased; after 48 hr the intensity

of D-glucose increased. No change in siutation was observed after 72 hr and also upon heating.

Mimonoside B. On precipitation, a light buff amorphous powder was obtained, m.p. 240-42°C (dec.); IR (KBr, cm^{-1}); 3400-2750 (broad), 1735, 1650, 1430, 1385, 1365, 1250, 1085, 1030, 900 and 840; FAB MS: m/z 1323 $(M+H)^+$, 1191 $[(M+H)-132]^+$, 1177 $[(M+H)-146]^+$, 1059 $[(M+H)-(2\times132)]^+$, 1045 $[(M+H)-(146+132)]^+$, 1015 $[(M+H)-(146+162)]^+$, 913 $[(M+H)-(146+2\times132)]^+$, 751 $[(M+H)-(146+2\times132+162)]^+$, 619 $[(M+H)-(146+3\times132+162)]^+$, 457 $[(M+H)-(146+2\times132+2\times162)]$, 439, 249, 236, 205, 204, 190, 161, 134, 132, 95, etc. Acid hydrolysis furnished oleanolic acid, m.p. 305° (same as genin of mimonoside A). The aqueous hydrolysate revealed the presence of D-glucose, (R_f 0.12), L-arabinose (R_f 0.18), D-xylose (R_f 0.20) and L-rhamnose, (R_f 0.32)[10]. On basic hydrolysis mimonoside B remained as such indicating the absence of ester linkage.

Mimonoside C. Elution with methanol-chloroform (6:4) gave a light brown solid which was purified by dissolving in methanol and precipitating with chloroform repeatedly. Finally a white amorphous powder, m.p. 252-54°C (dec.) was obtained; IR (KBr, cm^{-1}): 3400 (broad), 2920, 2880, 1745, 1650, 1480, 1365, 1250, 1085, 1030, 960 and 870; FAB MS: m/z 1485 $(M+H)^+$, 1339 $[(M+H)-146]^+$, 1207 $[(M+H)-(146+132)]^+$, 1193 $[(M+H)-(2\times146)]^+$, 1075 $[(M+H)-(146+2\times132)]^+$, 1061 $[(M+H)-(2\times146+132)]^+$, 1031 $[(M+H)-(2\times146+162)]^+$, 929 $[(M+H)-(2\times146+2\times132)]^+$, 767 $[(M+H)-(2\times146+2\times132+162)]^+$, 635 $[(M+H)-(2\times146+3\times132+162)]^+$, 473 $[(M+H)-(2\times146+3\times132+2\times162)]^+$, 455, 437, 391, 265, 247, 205, 202, 134, 121, 119, 97, 91, etc. Acid hydrolysis afforded an aglycone, m.p. 298-301° (acetate: m.p. 265-67°) which was characterized as machaerinic acid[13] (m.m.p., IR, mass). The aqueous hydrolysate was concentrated and analysed on PC[10] which indicated the presence of D-glucose (R_f 0.12), L-arabinose (R_f 0.18), D-xylose (R_f 0.20) and L-rhamnose (R_f 0.32).

On basic hydrolysis the aqueous layer showed a spot due to L-rhamnose on PC and the BuOH layer gave a prosapogenol on column chromatography, m.p. 244-45°, indicating the presence of ester linkage. This prosapogenol on Kiliani hydrolysis[15] gave the same results as in the case of saponin A showing that the position of sugars in sugar chain were the same in both cases (mimonoside A and C).

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