Saponins and Sapogenins XVIII—Isolation of Proceric Acid, a New Triterpenic Acid, from Maharashtrian *Albizzia procera* Seeds

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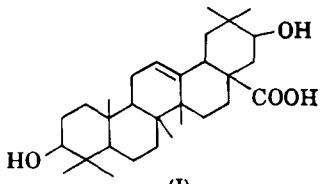
A colorless saponin isolated from Maharashtrian *Albizzia procera* seeds on hydrolysis yields a new pentacyclic triterpenic acid, m.p. 268–270°, acetate, m.p. 288–290°, methyl ester, m.p. 224–225°, and acetyl methyl ester, m.p. 280–281°. The compound has been named proceric acid.

THE ALBIZZIA PROCERA, Benth. seeds, obtained from Madhya Pradesh, were studied earlier in these laboratories (1, 2) for their saponin and sapogenin contents. They contained a saponin, proceranin, which on hydrolysis yields an acid genin identified as machaerinic acid (3β, 21β, dihydroxy Δ^{12}-18β-oleanene-28-oic acid) (I). To carry out additional work on proceranin, a supply of *Albizzia procera* seeds was obtained from the Silviculturist, Maharashtra, Poona. But surprisingly, when the genin obtained from these seeds was acetylated in the usual manner, the acetate obtained was quite different from the machaerinic acid acetate. Its melting point and rotation differed considerably from the acetates of all known triterpenic acids. Therefore, a detailed study of the genin from these seeds was instigated.



(I)

The well-defatted seed powder was extracted with alcohol, and the usual treatment gave a colorless product which passed all tests for saponin. The saponin, on hydrolysis with sulfuric acid, gave a colorless sapogenin which was further purified by potassium salt formation and crystallization, m.p. 268–270°. The acid genin was then transformed into a methyl ester with diazomethane, yielding colorless needles, m.p. 224–227°. The genin, on acetylation with cold pyridine and acetic anhydride, gave an acetate, m.p. 288–290°. The acetylation of the methyl ester with cold pyridine and acetic anhydride easily gave the acetyl methyl ester, melting at 281–282°. But the methylation of the acetate with diazomethane did not yield an acetyl methyl ester. All these compounds gave a yellow color, with tetranitromethane showing the presence of at least one carbon-carbon double bond.

Although the methyl ester and acetyl methyl ester have the same melting points as those of the corresponding derivatives of machaerinic acid, the melting points of the acetate and the genin differed considerably (Table I). The rotations of all these derivatives also differed.

The infrared spectra of the acetate showed the presence of an acetyl group at 8.05 μ and of a γ-lactone at 5.68 μ, which showed that the second OH group is not acetylated and has formed a lactone with the acid group. The I.R. spectra of the methyl ester also differed from the spectra of the methyl ester of machaerinic acid. The infrared spectra of this acid (proceric acid), when taken in pyridine under the conditions described (3), showed characteristic bands in the B range at 1332, 1292, and 1249 cm.$^{-1}$. This does not fit well into the oleanolic acid or in the ursolic acid scheme but is similar to the spectra of Δ^{12}-28-acids, as other positions of the double bond or the carbonyl groups give rise to storing alterations of the mentioned triplet. The NMR spectra (Fig. 1) was also different. An 18α-structure for this acid might be possible. Since this acid may be new, it has been tentatively named proceric acid.

The NMR spectrum has the vinylic hydrogen absorption at 62–65 cycles per second (c.p.s.), which is rather strange. Usually such an absorption falls in the 75–79-c.p.s. region. That the compound shows low absorption may mean that the double bond is conjugated. For example, if there is a ketone at C-11 in addition to the double bond at C-12, such an absorption is obtained. It may be possible that the double bond is not at C-12 according to the findings of NMR spectrography.

The low absorption from 105–120 c.p.s. represents the alpha hydrogen of the C-3 acetate function. This absorption is about where it should be.

The doublet at 127.8 and 133.9 c.p.s. probably represents the alpha hydrogen to the alcoholic oxygen of lactone. But again this absorption is too high, *i.e.*, upfield. For example, in dumortierigenin diacetate, where a five-membered lactone is present (4), the alpha hydrogen occurs at 104–120 c.p.s. The peak at 211.5 c.p.s. represents the methyl group of the acetate function at C-3.

The peaks at 246.6, 250.4, 253.2, 255.7, and 259.6 c.p.s. represent saturated methyl functions. In other words, there is no vinylic methyl group of the type C:C $-CH_3$. The smaller peaks at the right-hand side of the spectrum 187.6, 198.7, 225.6 c.p.s., etc., stand for methylene or methine hydrogens.

This shows that the present genin acetate is a lactone between second OH and acid group. The 18α-structure for this acid, on the study of the molecular model and I.R. spectra, is not ruled out.

EXPERIMENTAL[1]

Defatting.—Well-powdered seeds (250 Gm.) were exhausted in a Soxhlet apparatus with light petroleum ether (40–60°). Recovery of the solvent gave a greenish-yellow oil with the typical odor of *Albizzia* seed oils.

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[1] All melting points were taken on a Kofler hot microscopical stage and are corrected. The I.R. spectra were recorded on an Infracord model 137 and a Perkin-Elmer model 221 spectrophotometer by I. P. Varshney and Professor R. Tschesche, respectively. The NMR spectra were recorded by the Department of Chemistry, Whitmore Laboratory, The Pennsylvania State University. The rotations are in chloroform solution.

TABLE I.—MELTING POINTS

	Machaerinic Acid, m.p.	$[\alpha]_D$	Proceric Acid, m.p.	$[\alpha]_D$	Mixed M.p.
Genin	256–258	+82.4	268–270	...	263–267
Acetate	258–260	+88	288–290	+16.67	260–262
Methyl ester	224–225	+80.7	224–227	+67.16	225–227
Acetyl methyl ester	278–280	+90	281–282	+80.20	282–283
Acetyl bromolactone	276–278	+80	...	...	...

Extraction.—The defatted seed powder was exhausted with 95% alcohol in a Soxhlet apparatus, and the extract was concentrated to a brown syrupy liquid, which was extracted successively with petroleum ether, ether, chloroform, carbontetrachloride, and acetone to remove the impurities soluble in these solvents. The residue left was then dissolved in ethyl alcohol and filtered. The solution was added dropwise to a large amount of ether when a light brown precipitate was obtained. This operation of dissolution in alcohol and precipitation was repeated several times; finally, the precipitation occurred in acetone. The final precipitate was taken in alcohol and treated with activated charcoal. It was again precipitated in acetone. This gave a light cream-colored hygroscopic powder of saponin. This product gave all the tests for saponins—sneezing, abundant foam on shaking with water, toxicity to fishes in low concentrations, strong hemolytic effect in dilute concentrations, and the specific color reactions (5).

Isolation of Sapogenin.—The cream-colored powder of the saponin obtained by the ether/acetone precipitations was dissolved in a large amount of water and hydrolyzed with sulfuric acid (5–7%) by heating the solution first on a boiling water bath for 1 hour, then completing the hydrolysis by refluxing the solution for 1 more hour. After completion of the hydrolysis, the precipitate was filtered, washed with water until the washings were neutral, and finally dried. The genin thus obtained was dissolved in alcohol and decolorized with activated charcoal.

The crude genin was converted into potassium salt by heating with an alcoholic solution of caustic potash for 1 hour and thereafter distilling off half of the solvent. The solution was diluted with a large quantity of water and extracted three times with ether. The ethereal extracts were combined and washed free of alkali. On recovery of the solvent, an appreciable quantity of the neutral sapogenin was not obtained. The alkaline solution left after ether extraction was acidified with hydrochloric acid and left for 2 hours. The precipitate obtained was filtered and washed with water free of hydrochloric acid and crystallized from methanol, giving the acid genin, m.p. 268–270°.

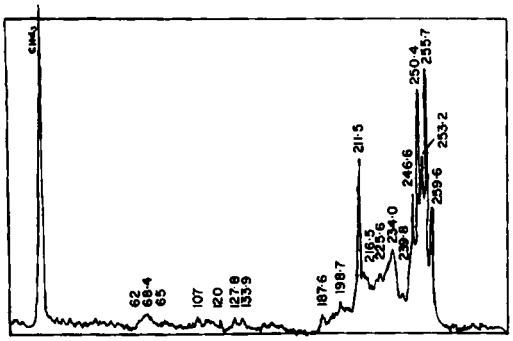

Fig. 1.—To convert to standard T values, divide by 40, then add 2.75 in every case. At 40 megacycles (Varian)—C_2Cl_4, solvent; $CHCl_3$, standard (internal).

Acetylation.—The acid genin was acetylated with acetic anhydride in the presence of pyridine by keeping the mixture overnight. It was poured in ice-water when the acetate was precipitated and filtered. The precipitate, after washing free of acid and pyridine, was crystallized as needles from methyl alcohol, m.p. 288–290°. It gave a positive reaction with tetranitromethane, $[\alpha]_D$ = +16.67°.

Anal.—Calcd. for $C_{32}H_{48}O_4$: C, 77.37; H, 9.74. Found: C, 77.08 and 77.52; H, 9.36 and 9.56.

Deacetylation.—Three-hundred milligrams of the acetate, m.p. 288–290°, was refluxed for 2 hours with methyl alcoholic potassium hydroxide (60 ml., 5%). The solution was diluted with a large amount of water and left overnight at room temperature, but no solid potassium salt separated out. The solution was acidified with hydrochloric acid which gave the precipitate of the acid sapogenin. The precipitate was filtered and washed with water free of acid and crystallized from methanol, m.p. 268–270°. It produced a yellow color with tetranitromethane and a positive Liebermann-Burchard reaction.

Anal.—Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.23. Found: C, 76.43; H, 10.63.

Methylation.—The acid genin (100 mg.) was dissolved in ether, and an excess of the ethereal solution of diazomethane was added to it. The solution was left overnight, and the excess of diazomethane was then removed on a water bath. The residue left, after the evaporation of the ether, was crystallized as needles from methanol, m.p. 224–227°. This showed unsaturation with tetranitromethane, $[\alpha]_D$ = +67.16°.

Anal.—Calcd. for $C_{31}H_{50}O_4$: C, 76.49; H, 10.36. Found: C, 75.68; H, 10.38.

Acetyl Methyl Ester.—The methyl ester (100 mg.) was acetylated with acetic anhydride and pyridine in the usual manner. It crystallized as colorless needles from methyl alcohol, m.p. 281–282°, $[\alpha]_D$ = + 80.20°.

Anal.—Calcd. for $C_{35}H_{54}O_6$: C, 73.64; H, 9.53. Found: C, 74.00; H, 9.57.

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