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CHEMICAL CONSTITUENTS OF PROSOPIS GLANDULOSA

Part I.—Isolation of Prosopol, Prosopenol and β -Sitosterol

M. MANZOOR-I-KHUDA,* S. A. ABBAS and W. A. ZAIDI

Drugs, Pharmaceuticals and Pesticides Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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Two new crystalline substances designated as prosopol, m.p. 83.5°C, and prosopenol, m.p. 245°C, have been isolated from *Prosopis glandulosa*. β -Sitosterol, m.p. 136°C, has been isolated and identified.

Prosopis glandulosa is a plant abundantly available in and around Karachi. No chemical investigation on this plant has so far appeared in the literature. The aerial part of the fresh plant material on extraction with boiling benzene followed by column chromatography of the benzene extractive gave prosopol, m.p. 83.5°C, $\alpha_D^{20} - 1^\circ$ (1%, CHCl_3). It analysed for $\text{C}_{29}\text{H}_{56}\text{O}$ and showed the presence of at least one (C) CH_3 group. It formed an acetate, $\text{C}_{31}\text{H}_{58}\text{O}_2$, m.p. 67-8°C, $\alpha_D^{34} - 24^\circ$ (0.61%; CHCl_3). From a comparison of available data for long chain fatty alcohols (Table I), prosopol is indicated to be a new alcohol. Prosopol does not decolorize dilute bromine solution in carbon tetrachloride, thus indicating absence of unsaturation in the molecule. It did not give any coloration with Salkowski and Liebermann-Burchard test. The molecular formula indicates presence of saturated cyclic rings in the molecule.

The fresh leaves of the plant on percolation with alcohol at room temperature gave an extractive which on concentration under reduced pressure gave mainly an aqueous substrate with suspended solid material. The petroleum ether (40-60°C) extractive of the aqueous layer was dried (Na_2SO_4) and concentrated, when a crystalline material A deposited. The soluble portion was subjected to column chromatography on alumina. The petroleum ether (40-60°C) elute

on rechromatography gave a small amount of prosopol, m.p. 82-83°C. The petroleum ether-benzene elute on rechromatography gave a material, m.p. 136°C, which gave a superimposable IR spectrum with β -sitosterol and the m.p. was undepressed on admixture with an authentic sample.

Investigations on the aqueous layer obtained from the plant extract are continuing, the aqueous ballast gives a positive test with Dragendorff's reagent.

TABLE I.

Alcohol	Mol. formula	M.p.	M.p. of acetate
1-Hexacosanol ¹	$\text{C}_{26}\text{H}_{54}\text{O}$	79°	65°
1-Heptacosanol ²	$\text{C}_{27}\text{H}_{56}\text{O}$	18-81.5°	44-46°
1-Octacosanol ³ (clutyl alcohol)	$\text{C}_{28}\text{H}_{58}\text{O}$	82.5°	64°
Prosopol	$\text{C}_{29}\text{H}_{56}\text{O}$	83.5°	67-80°
Cephalandrol ⁴	$\text{C}_{29}\text{H}_{58}\text{O}$	81.5-82.5°	71°
1-Nonacosanol ⁵	$\text{C}_{29}\text{H}_{60}\text{O}$	82.5°	43-43.5°
1-Triacontanol ⁶	$\text{C}_{30}\text{H}_{62}\text{O}$	86.5°	69°

*Present address: Technological Research Board, Jute Research Institute, PCJC, Dacca 15.

Crystalline precipitate A on further purification by charcoaling followed by several crystallisations from ethanol gave prosopanol, $C_{30}H_{50}O_3$, m.p. $245^\circ C$, $\alpha_D^{30} + 27^\circ$ (0.7, EtOH). This gave an acetate, m.p. $223-24^\circ C$, $C_{34}H_{54}O_5$, $\alpha_D^{34} - 18^\circ$ (0.9%, EtOH). Prosopanol gave a violet-purple colour with Libermann-Burchard test.

The IR absorption peaks at 3020i, 1690, 1650, 1415 and 885 cm^{-1} indicate the presence of an isolated double bond possibly of the vinyl type. It is confirmed by its absorption of one mole of hydrogen on microhydrogenation. In the UV absorption spectra it gave a rising curve with strong end absorption.

In the NMR spectra prosopanol gave peaks at 9.3, 9.1 (21 H; seven $C-CH_3$), 8.8, 8.7, 8.5, 8.34, 6.67, 6.46, 5.88 (2H; $=CH_2$), and 3.54τ (one H; OH). Prosopanyl acetate gave peaks at 9.1, 9.03, 9.0 (21 H; seven $C-CH_3$), 8.8, 8.66, 8.36, 8.0, (6H; two acetyl methyls), 6.35, 6.15, 6.0, 5.74, 5.48 ($=CH_2$) and 3.0τ (one H; OH).

The IR, UV and NMR studies show that prosopanol has 3 hydroxyl groups, one of which is placed in a tertiary or sterically hindered position thus allowing formation of only a diacetate from prosopanol. The acetate showed a total of 54 protons in the NMR spectra. These observations indicate prosopanol to be a $C_{30}H_{50}O_3$ compound,

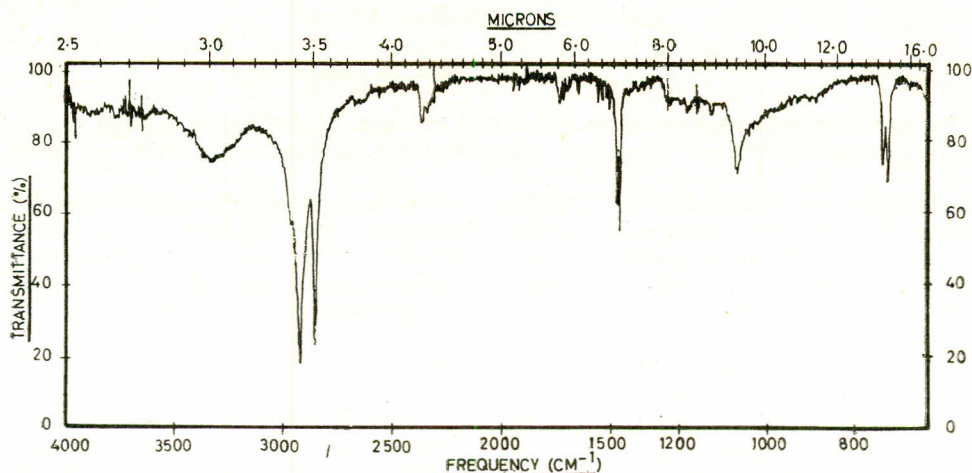


Fig. 1.—IR spectrum of prosopanol.

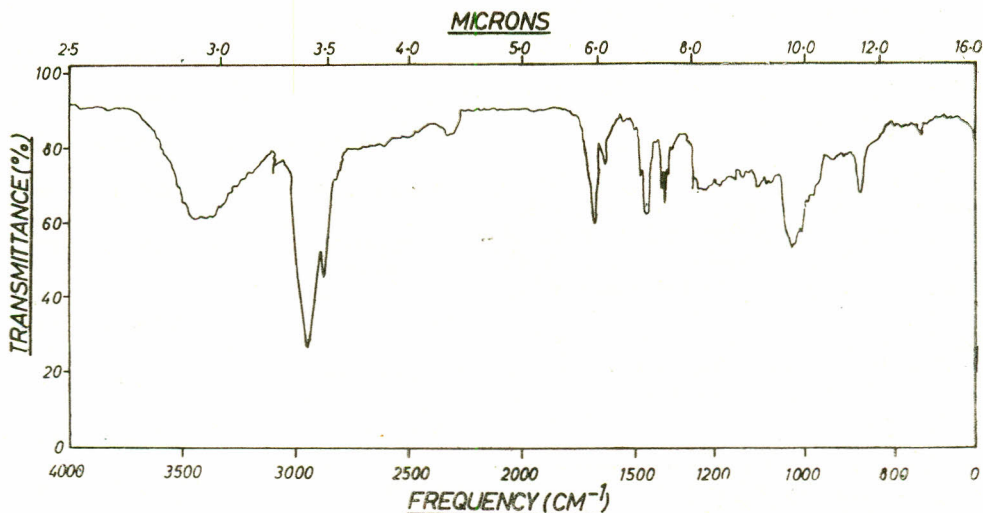


Fig. 2.—IR spectrum of prosopanol.

possibly a dihydroxy lupeol. Prosopanol is a hitherto unreported alcohol.

Experimental

The IR spectra were taken in KBr pellet on Perkin Elmer model 237 IR spectrophotometer, UV spectra were determined in ethanol on Beckmann Model DK-2 spectrophotometer, Optical rotation was determined in ethanol or chloroform with Schmidt-Haensch polarimeter. NMR spectra, with tetramethyl silane as external standard, were determined in CDCl_3 solution by Dr. S. H. Zaidi of Physics Division, Central Laboratories, PCSIR. All m. ps are uncorrected. The analyses were carried out by Drs. Pascher and Pascher, West Germany and Microanalytical Section, Central Laboratories, PCSIR.

Isolation of Prosopol.—Aerial part of the plant *Prosopis glandulosa* were collected in a fresh condition (380 g.; 50% moisture) and refluxed with benzene (700 ml) for 18 hr. This extraction was repeated once and the combined extracts were concentrated (50 ml) *in vacuo* and subjected to column chromatography on neutral alumina (250 g). The benzene elute was concentrated and allowed to cool when a substance, m. p. 82–83°C (1.11 g) was obtained which on repeated crystallisation from benzene gave *prosopol*, m. p. 83.5°, $\alpha_D^{30} - 1.0$ (1%, chloroform). (Found: C, 79.75; H, 13.90%. $\text{C}_{29}\text{H}_{56}\text{O} \cdot \text{H}_2\text{O}$ requires: C, 79.4; H, 13.35%). After drying *in vacuo* for 24 hr. Found: O, 4.25; (C)CH₃, 3.45%; mol. wt., 386.5. $\text{C}_{29}\text{H}_{56}\text{O}$ requires: O, 3.8; (C)CH₃ (for one) 3.55%; mol. wt., 420.7). The above molecular formula was confirmed by mass molecular weight determination, giving a value of 420. It had no UV absorption above 220 m μ in ethanol solution (~ 0.1%). A solution of *prosopol* in carbon tetrachloride did not decolorize dilute bromine solution in the same solvent. It had ν_{max} (KBr) 2920, 2852, 1470, 1460, 1065, 780 and 770 cm^{-1} . No coloration was observed during Salkowski and Liebermann-Burchard test on *prosopol*.

Isolation of β -Sitosterol.—The fresh leaves of the plant (5.5 kg, 50% moisture) were soaked in ethanol (22 l.) for 3 days and the ethanolic extract percolated out. This extraction was repeated three times and the total extract was concentrated under reduced pressure in a cyclone evaporator. The mainly aqueous concentrate (2 l.) was extracted with petroleum ether (40–60°C) and some insoluble materials were removed by filtration. The petroleum ether extract was dried (Na_2SO_4) and concentrated to give some semicrystalline material A (3.2 g),

and a petroleum ether-soluble fraction which was chromatographed on neutral alumina. The column was successively eluted with petroleum ether, benzene-petroleum ether, benzene, benzene-ether, ether, ether-chloroform, chloroform and chloroform-methanol. The petroleum ether elutes gave a small quantity of substance which on crystallisation from benzene gave *prosopol*, m. p. 82–3°C.

The above experiment was repeated with 2 kg of the leaves whence after the above working the petroleum ether-benzene elute was rechromatographed on alumina, and the petroleum ether-benzene elute was crystallised from methanol to give β -sitosterol (0.7 g), m. p. 136°C, $\alpha_D^{30} + 38^\circ$ (0.5%, EtOH). (Found: C, 81.7; H, 11.6; O, 6.45. $\text{C}_{29}\text{H}_{50}\text{O} \cdot 0.5\text{H}_2\text{O}$ requires: C, 82.2; H, 12.1; O, 5.7%). Its IR spectrum was identical with that of an authentic specimen of β -sitosterol, and the mixed m. p. was undepressed.

Isolation of Prosopanol.—Fraction A on charcoaling followed by repeated crystallisation from ethanol gave *prosopanol*, m. p. 245°C, $\alpha_D^{32} + 27^\circ$ (0.7%, EtOH). (Found: C, 77.1, 77.35; H, 10.80, 10.85; O, 12.15%. $\text{C}_{30}\text{H}_{50}\text{O}_3 \cdot 0.5\text{H}_2\text{O}$ requires: C, 77.05; H, 11.0; O, 12.0%. Sample dried at 100°C for 24 hr *in vacuo*, Found: C, 78.6; H, 10.65%. $\text{C}_{30}\text{H}_{50}\text{O}_3$ requires: C, 78.55; H, 10.95%.) It had no maximal UV absorption above 220 m μ in ethanol solution (0.1%) but gave a rising curve ~ 260 m μ downwards, with a strong end absorption below 220 m μ . On microhydrogenation in glacial acetic acid over Pt at 25°C, it absorbed 1.2 moles of hydrogen showing the presence of at least one double bond. It had ν_{max} (KBr) 2950, 2878, 1682, 1637, 1480, 1450, 1385, 1395 and 880 cm^{-1} . *Prosopanol* gave a violet-pink colour when subjected to Liebermann-Burchard test (chloroform solution-acetic anhydride-conc. sulphuric acid).

Prosopyl Acetate.—*Prosopyl* (0.2 g) was refluxed with acetic anhydride (10 ml) containing a few drops of acetyl chloride for 1 hr. The mixture was allowed to cool after dilution with water, when a crystalline solid separated, which was filtered, washed with water, dried *in vacuo* (0.18 g) and recrystallised from petroleum ether. The crystals on column chromatography over neutral alumina (4.5 g) followed by crystallisation from petroleum ether (40–60°C) gave *prosopyl acetate*, m. p. 67–68°C. $\alpha_D^{30} - 24^\circ$ (0.61%, CHCl_3). (Found: C, 80.1; H, 13.3; O, 6.6; O-acetyl, 8.3%. $\text{C}_{31}\text{H}_{58}\text{O}_2$ requires: C, 80.45; H, 12.7; O, 6.9; one O-acetyl, 9.3%)-

It had ν_{\max} (KBr) 2950, 2880, 1730, 1710, 1620, 1478, 1462, 1450, 1385, 1370, 1360, 1240 and 888 cm^{-1} .

Prosoperyl Acetate.—Prosopanol (62 mg) was dissolved in acetic anhydride (2.0 ml) containing a few drops of acetyl chloride and left overnight at room temperature. The solvent was removed under reduced pressure and the residue (72 mg) crystallised from methanol to give *prosoperyl acetate*, m.p. 223–224°C, $\alpha_D^{34} - 17^\circ$ (0.89%, (EtOH) (Found: C, 75.45; H, 10.55 %, mol. wt. Rast. 500. $\text{C}_{34}\text{H}_{54}\text{O}_5$ requires: C, 75.4; H, 10.05%; mol wt. 543). It had ν_{\max} (KBr) 2920, 2855, 1740, 1470, 1460, 1240, 1170, 1155, 780 and 770 cm^{-1} .

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