Pithecolobium dulce I

Isolation and Characterization of Legume Constituents

By S. K. NIGAM, R. K. GUPTA†, and C. R. MITRA

The seed and the mesocarp of the *P. dulce* legumes were worked up separately. The alcoholic extract of the seed powder yielded (I) a saponin, m.p. 175–181°; sapogenin provisionally named pithogenin, $C_{28}H_{44}O_4$, m.p. 207–208°; $[\alpha]_D^{17°}$ + 81°; elemental analyses and the spectrophotometric data suggest it to be a steroid genin; (II) a sterol-glucoside-B, m.p. 276–278°; the aglycone, m.p. 159–160°; and (III) a flavone, m.p. 298–306°. The seed fat (20%) similar to the leguminous fats in general, was purified and refined and the characteristics noted. The seed also yielded pure lecithin (0.7%). The sweet pulpy mesocarp yielded hexacosanol and a sterol-glucoside-A, m.p. 282–286°; aglycone, m.p. 136–138°. The sugar (66.5%; mostly glucose) and the amino acids, L-proline, L-leucine, L-valine, and asparagine were present.

PITHECOLOBIUM DULCE, Benth., N. O. *Leguminosae* (habitat: Mexico and Deccan peninsula of India), is extensively cultivated as a hedge plant. The shrub is hardy and stands frequent pollarding. It is reported in folk medicine to be useful in the treatment of leprosy (1). A few species of this genus are toxic. Alkaloidal principles have been isolated from *P. saman* (2). The bark and beans of another species, *P. lobatum* (3), are reported to cause dysuria and colic. As no work appears to have been carried out with the *P. dulce* legume, systematic chemical examination of the legumes was undertaken.

The ripe legumes consist of six to eight hard, black, flattened seeds embedded in a sweet pulpy mesocarp. The mesocarp appears to be edible, but it is reported to cause colic and gives a tickling sensation to mouth mucosa.

Constituents of Mesocarp

The residue from the alcohol-miscible free-flowing syrup, obtained from the mesocarp on extraction with nonpolar solvents, yielded a waxy fraction and a sterol-glucoside-A, m.p. 282–286° in yields of 0.8% and 0.02%, respectively. The fatty alcohol constituting the major portion of the waxy fraction was identified as hexacosanol. The sterol-glucoside-A yielded on hydrolysis an apparently new sterol-aglycone-A, m.p. 136–138°; the sugar moiety of the glucoside has been identified as glucose through paper co-chromatography in two solvent systems (*n*-butanol 4, acetic acid 1, water 5; and pyridine 1, ethyl acetate 2, water 2; aniline phthalate spray). The dextrorotation and other characteristics of the sterol-aglycone-A (Table I) differ from those of the sito-sterols and so also of the parent sterol glucoside-A from the β-D-glucoside of β-sitosterol (4).

The alcohol-soluble fraction of the mesocarp, when examined in aliquot portions, showed the presence of 66.5% of total sugar of which reducing sugar [estimated as glucose; iodometric (5)] was found to be 62.9% of the alcohol-soluble total solid. The paper chromatograms in two solvent systems (same as above) showed the presence of two spots of glucose and fructose but no sucrose was indicated. The alcoholic extract of the mesocarp yielded an osazone identical with authentic glucosazone. The amino acids, L-proline, L-leucine, L-valine, and asparagine were identified by paper chromatography (*n*-butanol 4, acetic acid 1, water 5; ninhydrin spray) while an additional spot in the chromatogram remained unidentified (6). The more soluble (aqueous) fraction of the alcoholic extractive showed the presence of traces of a saponin and a flavone.

Constituents of the Seed

In addition to 20% fixed oil, an appreciable quantity of a saponin (*ca.* 2.4%) as well as lecithin (*ca.* 0.7%) and a flavone (*ca.* 0.0001%) have been isolated from the seed for the first time. Generally, leguminous plants do not bear saponins, the subfamily, *Papilionoideae* (7) and the genera, *Albizzia* (8) and *Acacia* (9) being a few exceptions. An unidentified saponin has so far been indicated to be present in only one species of this genus, *P. cauliflorum* (10).

In order to isolate the different constituents of the seed under mild conditions and to avoid interference of the fat towards the isolation of the lipid-associates, such as phosphatides, powdered seed material was at first exhaustively percolated with alcohol and the fat in the residual meal was subsequently extracted with hydrocarbon solvent. The extractives from both the solvents were worked up separately. Concentration of the alcoholic extract deposited microcrystalline needles of sterol-glucoside-B, m.p. 276–278° (*ca.* 0.007%) which had glucose as the sugar component (confirmed through paper chromatography). The sterol-aglycone-B melted at 159–160°.

The mother liquor, freed of alcohol under reduced pressure, was macerated successively with excess of

Received July 30, 1962, from the Utilization Research Laboratory, National Botanic Gardens, Lucknow, India.
Accepted for publication October 3, 1962.
The authors thank Mr. J. Saran of C.D.R.I. for microanalyses, Dr. I. P. Varshney of Aligarh University for the infrared spectra, Mr. B. P. Mital for recording the ultraviolet data, and Professor K. N. Kaul, Director, National Botanic Gardens, for his interest in the investigation.
† Present address: Indian Veterinary Research Institute, Izatnagar, India.

TABLE I.—CHARACTERISTICS OF THE STEROLS OF *P. dulce* LEGUME

	Sterol-aglycone-A	Sterol-aglycone-B	Sterol from Phosphatidic Fraction
Melting point	136–138°	159–160°	159–160°
Optical rotation	+37°	−44°	...
I.R. (Nujol; cm.$^{-1}$)	3279, 2817, 2532, 1695, 1450, 1366, 1163, 1129, 1082, 1048, 966,	3380, 1713, 1641, 1600, 1323, 1254, 1216, 1197, 1155, 1141, 1103, 1067, 1047, 1040, 1018, 971, 939, 892, 869, 846, 832.	...
Acetate: m.p.	119°	176°	176°
$[\alpha]_D$	−16°	−16.4°	−16.0°
Benzoate: m.p.	192°	185°	185°

petrol-ether (b.p. 40–60°), ether, and ethyl acetate. Four well-defined fractions were obtained: (*a*) petrol-ether soluble, (*b*) petrol-ether insoluble and ether soluble, (*c*) ethyl acetate soluble but petrol-ether and ether insoluble and (*d*) alcohol-water soluble but insoluble in petrol-ether and ether. The dark green semisolid petrol-ether soluble fraction (*a*) on digestion with acetone yielded acetone-insoluble phosphatidic material, and the acetone solution yielded the glycerides. The phosphatidic fraction on usual purification and fractionation with alcohol yielded lecithin, $[\alpha]_D^{28°}$ + 14° (c, 1.0; $CHCl_3$); nitrogen, 1.0% (11), as the major component. The sterol, isolated from the unsaponifiable fraction of the phospholipids of the seed, was identical with the sterol-aglycone-B (Table I; the mixed melting points of both the sterols as well as of their acetates and the benzoates were undepressed). Obviously, the phytosterol present in the seed is different from that of the mesocarp. Further studies in the sterols are being continued.

The ether- and ethyl acetate-soluble fractions (*b*) and (*c*) yielded a very small quantity of a flavone melting at 298–306°; $\lambda_{max.}^{EtOH}$ 270, 349 mμ, $\lambda_{min.}^{EtOH}$ 248 mμ; color tests: magnesium hydrochloric acid, orange-red; ferric chloride, dark olive-green; and lead acetate, bright lemon-yellow; its acetate melted at 208–209°. The paper chromatogram (*n*-butanol 5, acetic acid 1, water 4) of the flavone confirmed it to be a single substance.

The dilute alcohol-soluble fraction (*d*) of the seed extractive manifested considerable frothing on removal of most of the solvent under reduced pressure and on usual working. This fraction yielded a saponin as a white foam, m.p. 175–181°; $[\alpha]_D^{25°}$ + 48.5° (c, 1.0; 60% alcohol). Graded hydrolysis with 2–6% hydrochloric acid of the saponin gave the genin which, after purification through and regeneration from its acetate, melted at 207–208°; $[\alpha]_D^{17°}$ + 81° (c, 1.0; $CHCl_3$). Persistent difficulty was encountered in isolating the genin in workable yield by conventional hydrolysis of the saponin. Mild hydrolysis even at stages did not give satisfactory results. The crude genin was mostly resinous and repeated attempts for purification through chromatography using different eluents met with little success. Crude genin acetate was, therefore, prepared under mild conditions and purified through chromatography. In either case, the ultimate yield of the genin was very poor. However, the purity of

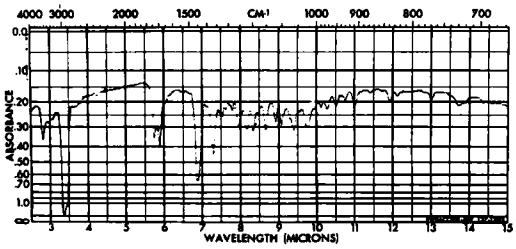

Fig. 1.—Spectrophotometric data of pithogenin.

the genin obtained through the acetate was ensured by repeated chromatography. A tentative molecular formula, $C_{28}H_{44}O_4$, was arrived at through elemental analyses of the genin and its acetate. The genin, provisionally named pithogenin, is LB-positive (pink to violet); with thionyl chloride-ferric chloride it develops orange to red; with thionyl chloride and antimony trichloride, a yellow to orange color (12). Spectrophotometric data of pithogenin [infrared bands at 3569, 2959, 2882, 1742, 1701, 1453, 1370, 1009, 988, 973, 951, 928, 907, 854, 839, cm^{-1} (Fig. 1); ultraviolet absorption (Fig. 2), $\lambda_{inflex.}^{EtOH}$ 260 mμ (ε, 772.4)] indicate the presence of hydroxyl and an isolated carbonyl group which suggest a similarity with steroid sapogenins (13, 14); further correlating data are being collected. The sugar moiety of the saponin was found to be gluclose identified through paper co-chromatography and confirmed through the mixed melting point of its osazone with glucosazone.

The seed fat obtained as a greenish oil was processed free from alcohol-soluble material, refined and bleached (15), and showed the following characteristics: color (Lovibonds, 1-cm. cell), 1.1 Y; sp. gr. (26°), 0.9064; $n_D^{26°}$, 1.4635; acid No., 0.2; sap. No., 183; iodine value (Wij's), 68; and unsaponifiable, 1.07%.

EXPERIMENTAL[1]

Examination of Mesocarp

Mature legumes used in this investigation were collected during May and June. Average weight of a legume was 25 Gm. and that of a seed, 110 mg.

[1] Melting points are uncorrected.

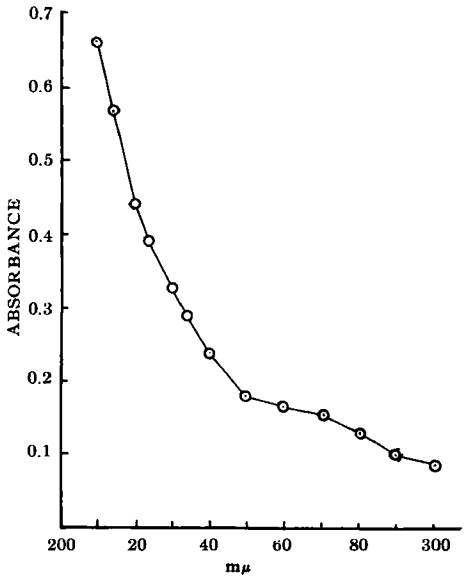

Fig. 2.—Ultraviolet absorption of pithogenin: $\lambda_{\text{inflex.}}^{\text{EtOH}}$ 260 mμ (ε, 772.4).

The seeds were separated from the pulpy mesocarp freed of brown brittle legume coat.

Sterol-glucoside-A.—Seventeen kilograms of air-dried sticky mesocarp was macerated and percolated with 21 L. alcohol five times until the alcohol insoluble material (*ca.* 10% of the air-dried mesocarp) was left behind mostly as fibrous residue. On removal of major bulk of alcohol by distillation under reduced pressure, a dark brown, syrupy, free-flowing mass was obtained which was successively extracted with 4 L. petrol-ether (b.p. 40–60°) three times, 4 L. ether three times, and 5 L. ethyl acetate twice. During this processing 3.1 Gm. sterol-glucoside-A separated out as microcrystalline shiny needles. It was further purified by crystallization from 2 L. of 90% alcohol and the final product melted at 282–286°, $[\alpha]_D^{25°}$ −20° (c, 1.0; pyridine).

Anal.—Calcd. for $C_{35}H_{60}O_6$: C, 72.9; H, 10.42. Found: C, 71.7; H, 10.7.

Sterol-aglycone-A.—Hydrolysis of 230 mg. of sterol-glucoside-A was effected by refluxing it with 75 ml. alcoholic hydrochloric acid (5% v/v) for 5 hours. After cooling the reaction mixture, the precipitated aglycone was taken up in ether and worked up. Its ether-petrol-ether mixture (90:10) was passed through a column of 5 Gm. alumina, and the resultant product on crystallization from alcohol-ether (90:10) yielded the sterol, m.p. 136–138°; $[\alpha]_D^{17°}$ + 37° (c, 1.0; $CHCl_3$).

Anal.—Calcd. for $C_{29}H_{50}O$: C, 84.07; H, 12.1. Found: C, 83.8; H, 12.2.

Sterol-aglycone-A-acetate.—The sterol aglycone-A (100 mg.) was dissolved in 1 ml. pyridine and refluxed with 2 ml. acetic anhydride for 8 hours at 110° and the reaction mixture worked up in the usual manner. The acetate was purified by passing its ether-petrol-ether (80:20) solution through a column of 5 Gm. alumina. When finally crystallized from alcohol it melted at 118–119°; $[\alpha]_D^{17°}$ − 16° (c, 1.0; $CHCl_3$).

Anal.—Calcd. for $C_{31}H_{52}O_2$: C, 81.53; H, 11.4. Found: C, 81.45; H, 11.2.

Sterol-aglycone-A-benzoate.—Fifty milligrams of the aglycone was taken up in 1 ml. pyridine and 2 ml. benzoyl chloride added dropwise. The reaction mixture was left at room temperature (20–35°) for 24 hours. The benzoate, on usual working and purification by passing its ethereal solution through a column of 4 Gm. alumina and on subsequent crystallization from alcohol, melted at 192°.

Anal.—Calcd. for $C_{36}H_{54}O_2$: C, 83.4; H, 10.4. Found: C, 83.1; H, 10.4.

Hexacosanol.—The ether, petrol-ether, and ethyl acetate soluble fractions, separately worked up, yielded 13.2 Gm. of waxy compound having a melting range of 65–75°. On repeated crystallizations from petrol-ether, hot alcohol, and alumina column chromatography using petrol-ether as eluent, the waxy compound yielded hexacosanol as a white microcrystalline product, m.p. 79°, mixed melting point with authentic sample 78–79°.

Anal.—Calcd. for $C_{26}H_{54}O$: C, 81.7; H, 14.1. Found: C, 81.5; H, 14.2.

Hexacosanol Acetate.—This was prepared with acetic anhydride and sodium acetate and after usual working and crystallization from alcohol it melted at 64°.

Anal.—Calcd. for $C_{28}H_{56}O_2$: C, 79.2; H, 13.2. Found: C, 79.3; H, 13.4.

Examination of the Seed

Powdered seeds weighing 8.8 Kg. were percolated at room temperature (15–30°) successively with 7 L. 95% alcohol four times and 7 L. petrol-ether (b.p. 60–80°) three times.

Sterol-glucoside-B.—The alcoholic percolate on concentration under reduced pressure below 50° deposited 0.8 Gm. microcrystalline sterol-glucoside-B which when recrystallized, as in the case of sterol-glucoside-A, melted at 276–278°; $[\alpha]_D^{25°}$ −25° (c, 1.0; pyridine).

Anal.—Calcd. for $C_{34}H_{58}O_6$: C, 72.6; H, 10.3. Found: C, 71.9; H, 10.2.

Sterol-aglycone-B.—Hydrolysis of 200 mg. of sterol-glucoside-B was carried out as in the case of sterol-glucoside-A to yield the sterol-aglycone-B which on usual purification and crystallization from alcohol melted at 159–160°; $[\alpha]_D^{36°}$ −44° (c, 1.0; $CHCl_3$).

Anal.—Calcd. for $C_{28}H_{48}O$: C, 84.0; H, 12.0. Found: C, 82.8; H, 12.0.

Sterol-aglycone-B-acetate.—Prepared in the usual way, the acetate on crystallization from alcohol melted at 176°; $[\alpha]_D^{34°}$ −16.4° (c, 1.0; $CHCl_3$).

Anal.—Calcd. for $C_{30}H_{50}O_2$: C, 81.4; H, 11.3. Found: C, 80.3; H, 10.6.

Sterol-aglycone-B-benzoate.—This was also prepared by heating the substance with benzoyl chloride in pyridine at 110° for 3 hours. The benzoate after repeated crystallizations from alcohol melted at 185°.

Anal.—Calcd. for $C_{35}H_{52}O_2$: C, 83.3; H, 10.3. Found: C, 82.7; H, 10.0.

The mother liquor after removal of the sterol-glucoside-B was further concentrated under reduced pressure when the resultant aqueous solution manifested copious frothing. It was ice cooled and extracted successively with 4 L. petrol-ether (b.p. 40–60°) three times, 4 L. ether three times, and 3 L.

ethyl acetate three times when finally a gelatinous mass was left in the aqueous phase. The petrol-ether soluble fraction obtained on removal of solvent finally *in vacuo* yielded 200 Gm. greenish residue which when repeatedly agitated with acetone, finally gave 54 Gm. acetone-insoluble phosphatidic material. The phospholipoid fraction was further extracted with cold alcohol and the residue, after removal of alcohol under reduced pressure, was purified by acetone and ether to yield 27.5 Gm. cream-white lecithin. The alcohol-insoluble phosphatidic material was then extracted with hot alcohol to yield a further quantity of 21.6 Gm. of alcohol soluble lecithin and 4.9 Gm. of alcohol-insoluble cephalin. Twenty-one grams of lecithin fraction on acid hydrolysis and subsequent saponification gave 0.8 Gm. of sterol, m.p. 160°. The acetate and the benzoate of the sterol were prepared as in the foregoing cases for comparison (Table I).

The ether and ethyl acetate soluble fractions yielded only the flavone.

Saponin and Pithogenin.—The gelatinous precipitate from the aqueous fraction of the seed extractive was dissolved in 300 ml. alcohol and 3 L. ether slowly added; the saponin precipitated out. On repeating the process with aliquot portions several times, 211 Gm. of the saponin was finally obtained as white foam, m.p. 175–181°, on removal of last traces of solvent *in vacuo*.

Saponin Acetate.—To a solution of 600 mg. of the saponin in 5 ml. pyridine, 25 ml. of acetic anhydride was added and the mixture left for 48 hours at room temperature (20–25°). The reaction mixture was next macerated with crushed ice and the precipitated saponin acetate was washed with water until free from acid. It was crystallized from alcohol and melted at 138°.

Pithogenin.—Ninety-four grams of saponin was hydrolyzed in 300 ml. of 90% alcohol with 2% hydrochloric acid at 20–35° for 24 hours. The cooled reaction mixture was extracted with 400 ml. of benzene. After separation of the benzene extract, the aqueous fraction was heated to 90° for 24 hours, cooled and extracted with benzene. The process was repeated five times with successive 1% increments in the acid percentage up to 6%. The combined benzene extract on usual working yielded 4.8 Gm. of crude genin as a dark brown product. Alumina column chromatography of 2.4 Gm. of genin using benzene-chloroform mixture (80:20) as the eluent yielded only 6 mg. of crystalline genin, melting at 195°, softening earlier.

Pithogenin Acetate.—This was prepared by heating 2.4 Gm. of the crude genin with 30 ml. of acetic anhydride and 8 Gm. of freshly fused sodium acetate on a steam bath for 72 hours. The reaction product was worked up in the usual manner. The genin acetate was eluted in a column of 20 Gm. alumina with ether-petrol-ether mixture (75:25) and six fractions were collected. The fourth and fifth fractions gave 120 mg. crystalline acetate which was further crystallized from ether-petrol-ether mixture (50:50) to yield a colorless acetate, m.p. 176–180°.

Anal.—Calcd. for $C_{28}H_{43}O_3 \cdot OCOCH_3$: C, 74.1; H, 9.5. Found: C, 73.8; H, 9.8.

Regeneration of Pithogenin.—Pithogenin was regenerated from 100 mg. of acetate with 10 ml. of 5% sodium carbonate solution at ordinary temperature. After usual working, 70 mg. of genin was obtained and purified by chromatography using ether-petrol-ether (85:15) as the eluent. Fifty milligrams of the product thus obtained were crystallized from ether-alcohol (90:10) to yield silky-white needles of pithogenin, m.p. 207–208°, $[\alpha]_D^{17°}$ +81° (c, 1.0; $CHCl_3$).

Anal.—Calcd. for $C_{28}H_{43}O_3OH$: C, 75.6; H, 9.6. Found: C, 75.3; H, 10.1.

REFERENCES

(1) Watts, G., "Dictionary of Economic Products of India," Vol. VI, Superintendent, Govt. Printing, Calcutta, 1893, p. 281.

(2) Wiesner, K., MacDonald, D. M., Volenta, Z., and Armstrong, R., *Can. J. Chem.*, **30**, 761(1953).

(3) Reimann, H. A., and Sukatoa, R. V., *Am. J. Med. Sci.*, **232**, 172(1956); through *Chem. Abstr.*, **50**, 17184(1956).

(4) Misra, G., Sharma, V. N., Mitra, C. R., and Kaul, K. N., *Soap, Perfumery Cosmetics*, **34**, 761(1961).

(5) Hinton, C. L., and Macara, T., *Analyst*, **49**, 2(1924).

(6) Airan, J. W., and Desai, R. M., *J. Univ. Bombay*, **34A**, 58(1953); through *Chem. Abstr.*, **48**, 10252(1954).

(7) Gedeon, J., *Arch. Pharm.*, **287**, 131(1954).

(8) Varshney, I. P., and Khan, Mohd., S. Y., *J. Sci. Res. Ind.*, **20B**, 30(1962).

(9) Wasicky, R., Ferreire, C., and Fonseca, E., *Anais Fac. Farm. Odontol. Univ. Sao Paulo*, **4**, 230(1944–1945); through *Chem. Abstr.*, **41**, 1474(1947).

(10) Altman, R. I. A., *Bolt. Tec. Inst. Agron. Norte (Belem, Brazil)*, **31**, 3(1956); through *Chem. Abstr.*, **52**, 506 (1958).

(11) Eckey, E. W., "Vegetable Fats and Oils," Reinhold Publishing Corp., New York, N. Y., 1954, pp. 52–57.

(12) Noller, C. R., Smith, R. A., Herris, G. H., and Walker, J. W., *J. Am. Chem. Soc.*, **64**, 3047(1942).

(13) Wall, M. E., Eddy, C. R., McClennan, M. L., and Klumpp, M. E., *Anal. Chem.*, **24**, 1377(1952).

(14) Wall, M. E., Krider, M. M., Rothman, E. S., and Eddy, C. R., *J. Biol. Chem.*, **198**, 533(1952).

(15) Awasthi, Y. C., Mitra, C. R., and Kaul, K. N., *Indian Oilseeds J.*, **6**, 38(1961).