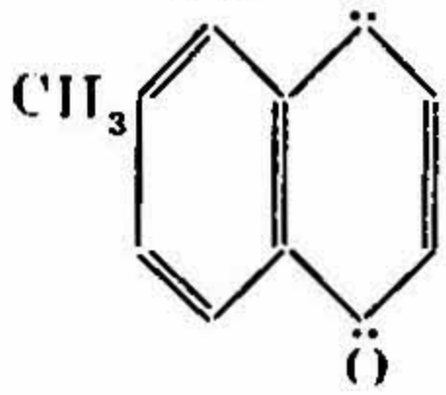
CHEMICAL EXAMINATION OF THE ROOT-BARK OF *PLUMBAGO ROSEA*, LINN.

By M. C. Tummin Katti and V. N. Patwardhan.

Chitramul (*Plumbago rosea* or *P. zeylanica*) is well known in Ayurvedic and Unani systems of medicine; the history, description, uses and the early literature up to 1889 have been given in *Pharmacographia Indica*, Vol. II, p. 328. Plumbagin was isolated from *Plumbago rosea* by W. Benttink in 1888 as a yellow, crystalline compound melting at 72° and having the molecular formula, $C_{16}H_{13}O_6$. In 1928 (*Proc. Ind. Sci. Congress*, 1928, **15**, 163) one of us (M.C.T.K.) communicated a preliminary examination of *Plumbago zeylanica* root, wherein the isolation of purified plumbagin (m.p. 78°) giving a benzoyl derivative melting at 146° was reported. Madinaveitia and Gallego (*Anal. Fis. Quim.*, 1928, **26**. 263) obtained plumbagin (m.p. 76°) from *Plumbago europea* and have described some of its properties and several derivatives. They are of opinion that plumbagin, to which they assign the formula $C_{11}H_8O_3$, is probably methyljuglone, the exact position of the methyl group being uncertain: it should therefore have one of the following three formulæ :—



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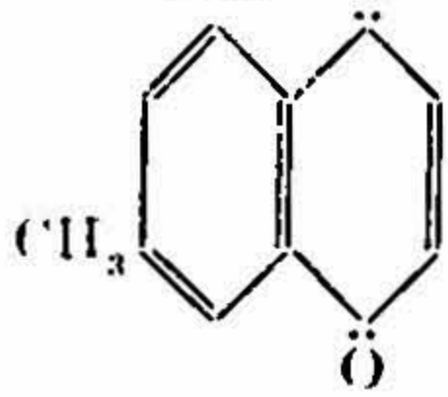

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Roy and Dutt (*J. Ind. Chem. Soc.*, 1928, **5**. 419) isolated plumbagin (m.p. 77–78°) from *Plumbago rosea* and *Plumbago zeylanica*, describing its properties and derivatives. Originally they assigned the formula $C_{18}H_{15}O_5$ to plumbagin, but later (*Chem. Ind.*, 1929, **7**. 40) changed it to $C_{18}H_{16}O_5$, when an objection was raised by Klein (*ibid.*, 1928, **6**, 1035) that the original formula did not satisfy the law of even atom numbers. From experimental evidence they are of opinion that plumbagin may be a lactone in addition to containing hydroxy- and para-quinone groupings.

Since it was apparent from the foregoing brief review that a systematic investigation of the drug is needed and that the information regarding the chemical nature of plumbagin itself is conflicting, it was deemed desirable to subject the root of *Plumbago rosea* to further examination.

Experimental.

The material employed for this investigation consisted of the dark brown root-bark obtained from the local market.

Preliminary examination : Test for alkaloids.—The ground material (15 g.) was digested with Prollius's fluid for two hours, when the filtrate, examined

in the usual manner for alkaloids, gave negative results. The disintegrated bark (100 g.) was accordingly distilled with steam and about 600 c.c. of the distillate collected ; an orange-red, oily material soon solidified on cooling and was filtered, the filtrate being extracted with ether. The solid was found to be plumbagin, and the residue from the ether extract consisted of plumbagin with some oily material while the colourless aqueous portion after extraction with ether was blank.

Extraction with solvents.—The powdered material (30 g.) was extracted in a Soxhlet apparatus successively with various solvents when the following percentages of extract dried at 100° were obtained :—Petroleum ether (b.p. 40–60°) 0.66, ethyl ether 0.47, chloroform 0.40, ethyl acetate 1.43, acetone 4.30, ethyl alcohol (90 per cent.) 11.23 ; total, 18.49.

The major portion of plumbagin came with petroleum and ethyl ether, while the extracts with chloroform, ethyl acetate and acetone contained only traces. The major portion of the acetone extract consisted of an amorphous, brown pigment, also found in the alcoholic extract which contained in addition a reducing sugar.

For a detailed examination the powdered root-bark (33 kg.) was thoroughly extracted with alcohol (90 per cent.). After removing the greater part of the solvent the syrupy, dark brown residue was well mixed with about 2 kg. of the alcohol-extracted material, dried completely and then extracted in a specially devised continuous extraction apparatus (Tummin Katti, *J. Ind. Chem. Soc.*, 1930, 7. 210) successively with low-boiling petroleum ether, ethyl ether, chloroform, ethyl acetate, acetone and ethyl alcohol.

Petroleum Ether Extract.

The solvent was dispersed and a portion of the dark residue steam distilled to separate plumbagin, but by this method all could not be removed. The residue after steam distillation was therefore extracted with ethyl ether and this, along with the ethyl ether solution of the remainder of the petroleum ether extract, repeatedly shaken with 3 per cent. sodium hydroxide solution, because when attempts were made to extract the ether solution first with sodium carbonate solution a very stable emulsion was usually formed. The alkaline extracts were immediately acidified and extracted with chloroform, the residue from which was distilled with steam.

Isolation of Plumbagin.—The resulting oily liquid soon solidified to an orange-red solid, crystallising from dilute alcohol in orange-yellow, silky needles melting at 75–76° ; a further quantity was recovered from the aqueous filtrate. The residue after steam distillation was an amorphous, brown solid from which nothing definitely crystalline could be obtained.

The ethereal solution after extraction with sodium hydroxide solution gave a viscous residue which was saponified with alcoholic sodium hydroxide. The alcohol was then distilled and the residual, strongly alkaline solution mixed with purified filter-paper pulp, dried and extracted with dry ethyl ether in a Soxhlet apparatus.

Examination of unsaponifiable matter.—The ethereal extract was washed with water and the solvent removed, when the residue, after crystallisation

four times from alcohol, gave colourless crystals melting at 135–136°. This compound gave the common colour reactions of phytosterols and yielded an acetate melting at 121–122°. The combined mother liquors were concentrated and allowed to cool, when a further crop of crystals was obtained; repeated crystallisation from alcohol yielded a compound melting at 137–138° giving the colour reactions of phytosterols and an acetate melting at 126–127°.

From their individual and mixed melting points (136–137°) the above phytosterols appear to be the same and identical with sitosterol; but the noticeable difference in the melting points of their acetates does not corroborate this assumption. The reported apparent difference in the melting point of sitosterol and of its acetate (*cf.* Anderson and Moore, *J. Amer. Chem. Soc.*, 1923, **45**. 1944: Anderson, *J. Amer. Chem. Soc.*, 1924, **46**, 1450; Schmid and Waschkau, *Monatsh.*, 1927, **48**, 139; Tummin Katti and Manjunath, *J. Ind. Chem. Soc.*, 1929, **6**, 839; Zechmeister and Tuzson, *Z. Physiol. Chem.*, 1929, **183**, 74; Tummin Katti and Shintre, *Arch. Pharm.*, 1930, **268**, 314; Jois and Manjunath, *J. Ind. Chem. Soc.*, 1930, **7**, 521; Tummin Katti and Puntambekar, *J. Ind. Chem. Soc.*, 1930, **7**, 221) is no doubt due to the fact that sitosterol is not homogeneous as formerly supposed, but a mixture of at least three isomeric sterols which differ in their physical properties (*cf.* Nabenhauer and Anderson, *J. Amer. Chem. Soc.*, 1926, **48**, 2972; Anderson and Shriner, *ibid.*, 1926, **48**, 2976 and 2987).

Isolation of arachidyl alcohol.—From the combined mother liquors after separating the above phytosterols, of which the last traces were removed by precipitation with digitonin, a compound melting at 71–72° after many crystallisations from alcohol was obtained. This compound dissolved in concentrated sulphuric acid, yielded an acetate melting at 57–58°, and seems to be identical with arachidyl alcohol isolated by Ameseder (*Z. Physiol. Chem.*, 1907, **52**, 121) from the fat of dermoid cyst. The material was insufficient for confirmatory work.

Examination of the fatty acids.—The soap after removal of unsaponifiable matter was dissolved in hot water which was filtered from the paper-pulp. The fatty acids obtained from the filtrate were separated as usual into saturated and unsaturated acids by Twitchell's method (*Ind. Eng. Chem.*, 1924, **13**, 806). The saturated fraction, after many crystallisations from alcohol, gave a product melting at 72–73° and having the molecular weight 364.5. It appears to be impure lignoceric acid, but the amount was insufficient for confirmation. The methyl esters of the unsaturated acids were fractionally distilled under reduced pressure (6 mm.), four fractions being collected as follows :—

Fraction	Temperature range	Iodine value	Saponification value
I	Up to 145°	91.0	289.8
II	155–161°	107.7	292.1
III	165–170°	115.0	295.6
IV	173–183°	114.1	298.1

The iodine and saponification values indicated that the first fraction contained mostly methyl oleate, and the remaining three fractions mixtures of

oleate and linoleate. The first fraction and the remaining three combined were separately hydrolysed and oxidised by cold alkaline permanganate solution. The hydroxy-acids thus obtained in each case were extracted in a Soxhlet apparatus successively with petroleum ether, ethyl ether and ethyl alcohol. Only a small amount of dihydroxystearic acid (m.p. 129°) could be obtained from the first fraction. From the last three fractions, an acid melting at 167–169° and having the molecular weight 347.2 was obtained in addition to dihydroxystearic acid. The molecular weight of this acid corresponds to that of a tetrahydroxystearic acid, but the melting point is slightly lower than that of sativic acid; a low melting point of tetrahydroxystearic acid has been observed before by several investigators, and it is therefore possible that this product is a mixture of isomeric acids having the formula, $C_{18}H_{36}O_6$.

Ethyl Ether Extract.

The solvent from the extract was removed and the residue distilled with steam, yielding only plumbagin; the residue was filtered and the filtrate extracted with chloroform. Attempts were made to dissolve the brown, water-insoluble residue in this chloroform solution, but with only partial success. The brown residue was amorphous, insoluble in water and only slightly soluble in ether or chloroform, but dissolved very readily in sodium hydroxide solution giving a dark red solution. Attempts to crystallise this material from alcohol and acetone failed.

The chloroform solution was extracted with ammonium carbonate and sodium hydroxide solutions; the former extract on acidification gave a very small amount of semi-solid material while the latter yielded more of the brown material. The chloroform solution after extraction with alkaline solutions was distilled, and the residue saponified with alcoholic sodium hydroxide solution, the dry soap being extracted in a Soxhlet apparatus with dry ethyl ether, a white crystalline material separating from the extractive.

Sitosterol-glucoside, a phytosterolin, $C_{33}H_{56}O_6$.—Concentration of the ether extract gave a further quantity of the white substance which, after being crystallised several times from alcohol, yielded a colourless product melting at 259–260°. It gave the usual colour reactions of phytosterols and this feature, coupled with its high melting point, suggested a phytosterolin. It was therefore hydrolysed with amyl alcoholic hydrochloric acid in the manner described by Power and Salway (*J.C.S.*, 1913, **103**, 404) in connection with the hydrolysis of ipuranol. The phytosterol thus obtained melted at 135–136° and was identical with that isolated from the petroleum ether extract before described. The aqueous acid liquid yielded glucosazone.

The ethereal filtrate after the separation of sitosterol-glucoside was concentrated and the residue after some time deposited some crystalline material which, on several crystallisations from alcohol, melted at 77–78° with previous softening. Its insolubility in concentrated sulphuric acid even after warming on the water-bath suggested that it might be a saturated hydrocarbon, but the quantity was too small for analysis.

Chloroform, Ethyl Acetate and Acetone Extracts.

The residue after removing the solvent gave a small amount of plumbagin on steam distillation; the water-insoluble residue consisted mostly of a

brown amorphous powder similar to that isolated from the ethyl ether extract. Ethyl acetate extracted only traces of plumbagin and the brown amorphous powder, while the acetone extract consisted almost entirely of the brown amorphous powder.

Ethyl Alcohol Extract.

The extract after removing the solvent was divided into water-soluble and water-insoluble portions, the latter consisting of brown material only. The former was precipitated with lead acetate solution and the precipitate de-leaded, when the filtrate from lead sulphide gave a green colour with ferric chloride solution indicating the presence of tannin. The filtrate from the lead precipitate was freed from excess of lead with hydrogen sulphide and concentrated under reduced pressure to a syrup, which reduced Fehling's solution abundantly and readily yielded an osazone melting at 204–205°, thus indicating glucose.

Properties of Plumbagin.

Further crystallisation did not raise the melting point of plumbagin, though a specimen isolated from *P. zeylanica* by one of us melted at 78°. The substance is freely soluble in ether, chloroform, benzene and alcohol, sparingly in cold water and moderately soluble in hot water; it dissolves in alkaline solutions giving a cherry-red colour. The original compound can be recovered if the alkaline solution is acidified immediately, otherwise the colour gradually changes to red and finally brown, the solution on acidification then yielding a brown, tarry material.

Plumbagin is volatile with steam and this property affords the best method of purification. Our analyses favour the empirical formula of Madinaveitia and Gallego (Found: C, 70.0 and 70.1; H, 4.1 and 4.3. $C_{11}H_8O_3$ requires C, 70.2; H, 4.3. $C_{18}H_{16}O_5$ requires C, 69.1; H, 5.1 per cent.). The molecular weight as determined by the freezing point depression method (in benzene) was found to be 182 (mean of 178, 188, 179 and 184), that calculated for $C_{11}H_8O_3$ being 188 and for $C_{18}H_{16}O_5$, 312.

Solutions of plumbagin give an intense red colour with ferric chloride solution indicating the presence of a phenolic group. Attempts to prepare the acetyl derivative were not successful, but the compound very readily yielded pale yellow needles of the benzoyl derivative when treated with benzoyl chloride in pyridine solution. Plumbagin does not contain a methoxy- or ethoxy-group; it yields a pale yellow carbethoxy-derivative melting at 108–109°.

An alcoholic solution of plumbagin when treated with bromine water gave an orange-red bromo-derivative which after three crystallisations from alcohol melted at 172–173° to a brown liquid and contained 44.4 per cent. of bromine. This compound is also obtained quantitatively when a saturated aqueous solution of plumbagin is treated with bromine water, and is evidently different from the dibromo-derivative of Roy and Dutt (*loc. cit.*) which melted at 122° and contained 34.4 per cent. of bromine.

Physiological properties.—Of all the compounds isolated from the drug during our work, only plumbagin and the brown powder appeared to possess any unusual physiological properties. The brown powder was found to be inert for all practical purposes by organoleptic tests. Plumbagin on the other

hand seemed to possess marked physiological activity. It has a very irritating odour and acts freely on the mucous membrane.

For testing its physiological activity, preliminary experiments were conducted to ascertain the minimum dose of plumbagin lethal to various experimental animals. The M.L.D. for albino rats was determined by injecting subcutaneously a 2 per cent. solution of the substance in 95 per cent. alcohol. The results are given in the following table :—

Weight of rat (grams)	c.c. injected	Result
216	1.00	Survived.[1]
278	1.00	Survived.[1]
253	1.00	Death after 2 days.
113	0.50	Survived.
105	0.50	Survived.
126	0.65	Survived.
70	0.35	Survived.
208	0.50	Survived.
102	0.50	Death after 18 hours.
82	0.50	Death after 10 hours.
57	0.35	Death after 15 minutes.[2]
68	0.40	Death after 40 hours.
82	0.50	Death after 20 hours.

[1] Urine was intensely yellow for a day and contained plumbagin.

[2] Intestinal puncture.

Three rats weighing roughly 100 grams each were injected with 0.5 c.c. of 95 per cent. alcohol for control, and all survived. The M.L.D. for rat seems to be between 110 and 120 milligrams of plumbagin per kilogram of body-weight. Similarly the M.L.D. for guinea pigs and frogs was found to be about 20 and 35 milligrams respectively per kilogram of body-weight. The number of animals used in these cases being small, the results could be taken as only indicative.

The post mortem examination of the animals in each case showed that plumbagin acted chiefly on the respiratory system. We hope to make a systematic pharmacological examination of this compound at a later date.

Summary.

The alcoholic extract of the root-bark of *Plumbago rosea* has been found to contain sitosterol, a fatty alcohol (probably arachidyl alcohol), oleic, linoleic and probably lignoceric acids, a phytosterolin (sitosterol-glucoside), a saturated hydrocarbon, glucose and a large amount of a brown, amorphous, inert material. Our analysis of plumbagin did not confirm the formula assigned to it by Roy and Dutt, but indicated that of Madinaveitia and Gallego as more probably being correct. Plumbagin seems to be the only compound in the root-bark responsible for the physiological activity of *Plumbago rosea*.

We wish to express our grateful thanks to Dr. C. V. Natarajan, Chemical Examiner to the Government of Mysore and Superintendent, Public Health Institute, Bangalore, for according us the facilities of the Public Health Institute Laboratories, and actively helping us in the methods and technique of animal experimentation. We also desire to record our appreciation of the keen interest shown by Professor Subrahmanyan in the present investigation.

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