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CHEMICAL EXAMINATION OF THE ROOT OF LASIOSIPHON MEISSNERIANUS

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The genus *Lasiosiphon* belongs to the natural order of *Thymelæaceæ*, which, although containing about 300 species, appears to afford but one drug that has received the official recognition of any of the national pharmacopœias, this being the mezereon bark, from *Daphne Mezereum*, Linné, and other European species of *Daphne*.

The plants of the above-mentioned natural order are mostly shrubs, a few of which are found in temperate regions of the Northern Hemisphere, but which are more common within the tropics, and occur most abundantly in South Africa and Australia. They are remarkable, among other characters, for the great tenacity of the inner bark, and, in many species, the latter possesses extremely acrid properties.

In a "Revised List of the Flora of Natal," compiled by J. Medley Wood, and published in the *Transactions of the South African Philosophical Society*, 1908, vol. xviii, Part 2, p. 218, twenty species of *Lasiosiphon* are enumerated, of which, however, several are unnamed, and only their approximation to other recognized species is indicated. In the list referred to, the following is recorded respecting the plant under present consideration:

"*L. Meisnerianus*, Endl., Var. Inanda, 1800 feet alt., *Wood*, 36; Van Reenen, 5–6000 feet alt., *Wood*, 4520; var, near Durban, *Wood*, 1028; var., *Gerrard* and *McKen*, 807; near Durban, *Wood*, 104, 529; without precise locality, Krauss, 237. Compare also De Candolle's *Prodromus*, vol. xiv, p. 594, where the specific name of the plant is evidently more correctly written *Meissnerianus*.

In a work by Andrew Smith, entitled "A Contribution to South African Materia Medica," third edition, 1905, there are several references (pp. 35, 77, 125) to the plant designated by him as *Lasiosiphon*

Meisneri—Kaffir, *isi-Dikili,* from which the following items of information respecting its characters and uses may be noted.

"The Lasiosiphons form a rather notable group. They have a heath-like appearance, with a tubular corolloid calyx, limb 5-parted. The flowers form a head with an involucre. The roots are very stringy and are used as sinnet. They are very scorching, if chewed, and will burn the tonsils and throat for twenty-four hours. Three species are used medicinally—*L. Meisneri; L. anthylloides;* and *L. linifolius.* The first of these is distinguished by its saffron or dark-orange flowers. Its leaves are three-quarters of an inch long, and less than one-eighth inch wide, hairy at the back. The involucral leaves are one-half inch long.

"*Lasiosiphon Meisneri* is a considerable bush. It is found in the lower basin of the Kat River, near its entrance into the Fish River, and is also found in various parts of Tembuland, being there used as a cure for snake-bite. The dose is from one-half to three-quarters of an ounce of the dried root, but some employ both leaves and root. The preparation is by infusion.

"It is somewhat difficult to say what its action in snake-bite precisely is, whether it is simply a powerful stimulant, almost blistering in its action, and long continued, and whether the same property does not explain the other uses of the plant. If a small fragment is chewed, it is nearly tasteless at first, but its burning quality is presently developed. Great caution must be used as to the quantity administered.

"*L. Meisneri* is also employed in cases of karroo fever, and a paste of the leaves for sores."

It has, furthermore, been noted by Smith (*loc. cit.,* p. 78) that "the root should always be used tolerably fresh, as it loses its virtue by long keeping."

One species of *Lasiosiphon,* namely, *L. eriocephalus,* Dcne., has been described in the "Pharmacographia Indica," vol. iii, p. 225. This is a native of the Deccan Peninsula and Ceylon, and is common on the hills of Western India. The plant is a shrub, with leaves like the willow, and the bark is a powerful vesicant, this property being attributed to a resinous constituent.

The most recent notice of the *Lasiosiphons* appears to be that contained in a short paper on South African Plants which was contributed by Mr. G. E. Oliver to the *Chemist and Druggist,* London, April 25, 1908, p. 645. It is there stated that these plants are much

esteemed among the natives for their tonic and blood-purifying properties, and also in the treatment of certain kinds of sore throat. The activity of the plant is said to reside chiefly in the root-bark, and with regard to the constituents of the latter Mr. Oliver has recorded the following observations: "A chemical examination of the root-bark shows it to contain a very small quantity of volatile oil, tannin (to which its virtue in sore throat would perhaps be attributable), and a resin, and it is apparently to this resin that its scorching properties are due, as it produces the sensation above referred to on the tongue, though it does not yield it to acidulated water when boiled with the latter. It contains no alkaloid."

EXPERIMENTAL.

The material used for this investigation consisted of the roots of the above-described plant, *Lasiosiphon Meissnerianus*, Endl., which had been kindly supplied by Mr. G. E. Oliver, of East London, Cape Colony, and was specially collected for the purpose.

The roots in question were more or less contorted and very irregular in size, some of the larger ones being as much as 10 centimetres (about 4 inches) in circumference. They were of a dark brown color, very rough and warty on the outer surface, and had a relatively thin bark, surrounding a lighter colored, very fibrous wood. As has previously been observed, when a little of the bark is chewed, a burning sensation is soon developed in the throat, which persists for several hours.

As a preliminary experiment, a small portion (10 grammes) of the ground material was tested for the presence of an alkaloid, but with a perfectly negative result.

Another portion (25 grammes) of the ground material was successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extracts, dried in a water-oven, were obtained:

Petroleum (b. p. 35–50°)	extracted.	0.20 Gm.	= 0.80 per cent.
Ether	"	0.45 "	= 1.80 " "
Chloroform	"	0.10 "	= 0.40 " "
Ethyl acetate	"	0.60 "	= 2.40 " "
Alcohol	"	2.10 "	= 8.40 " "
		Total, 3.45 Gm.	= 13.80 per cent.

For the purpose of a complete examination a quantity (29.94 kilogrammes) of the ground material was extracted by continuous percolation with hot alcohol, this operation having been kindly conducted by Messrs. Stafford Allen and Sons, of London. After the removal of the greater portion of the alcohol, a viscid, dark colored extract was obtained, amounting to 7.98 kilogrammes.

The whole of the above-mentioned extract was mixed with water, and distilled in a current of steam in a suitable apparatus for several hours, but no essential oil or other volatile product was obtained.

After the above operation there remained in the distillation apparatus a quantity of a dark brown resin and a dark colored aqueous liquid. The resin was separated by filtration, and well washed with hot water until nothing further appeared to be removed. The aqueous liquid and washings, on cooling, deposited a brown, resinous product, which was separately collected, and amounted to 320 grammes. A small portion (25 grammes) of this product was dissolved in alcohol, mixed with purified sawdust, and extracted in a Soxhlet apparatus with the following result:

Petroleum (b. p. 35–50°)	extracted,	*nil*	——
Ether	"	2.0 Gm.	= 8.0 per cent.
Chloroform	"	*nil*	——
Ethyl acetate	"	5.0 Gm.	= 20.0 per cent.
Alcohol	"	17.0 Gm.	= 68.0 per cent.
	Total,	24.0 Gm.	= 96.0 per cent.

These extracts were entirely resinous, and, although subjected to treatment both with acids and alkalis, nothing definite could be obtained from them.

The aqueous liquid, from which the resinous material had been completely removed, was concentrated to a small bulk, and shaken with ether, but only a trace of an amorphous product was thus obtained. On subsequently shaking the liquid with amyl alcohol a quantity of amorphous material was removed. This was heated with a 10 per cent. solution of sodium hydroxide, the alkaline liquid being then acidified and extracted with ether, but it yielded nothing definite.

The aqueous liquid was then treated with a slight excess of a solution of basic lead acetate, when an abundant brown precipitate

was produced. This was collected, well washed with water, then suspended in water, and decomposed with hydrogen sulphide. On filtering the mixture, and concentrating the filtrate, a resinous product was obtained which responded to the usual tests for tannic matter.

The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the lead, and the clear, filtered liquid evaporated to a small volume. It was found to contain a quantity of sugar, since it readily reduced Fehling's solution, and yielded *d*-phenylglucosazone, melting at 204–205°.

Examination of the Resin.

The resin which had been separated from the aqueous liquid, and thoroughly washed with hot water, as above described, amounted to 3685 grammes, thus corresponding to 12.3 per cent. of the weight of the drug. It was a brown, powdery substance, which, when inhaled, had an irritating effect on the nostrils, and when brought on the tongue, especially in alcoholic solution, a burning sensation was soon developed, similar to that produced on chewing the bark of the root.

For the examination of this resin a quantity (300 grammes) of it was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture then successively extracted in a Soxhlet apparatus with light petroleum (b. p. 35–50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This was a dark green, amorphous mass, amounting to 26 grammes. It was dissolved in alcohol, and heated for about four hours in a reflux apparatus with an alcoholic solution of potassium hydroxide. The alcohol was then removed, water added, and the alkaline mixture extracted with ether. The ethereal liquid was washed, dried, and evaporated, when a small amount of a crystalline substance was obtained, which separated from alcohol in plates, and gave the color reaction of the phytosterols. On recrystallizing the substance from a mixture of ethyl acetate and dilute alcohol it was obtained in the form of flat needles, melting at 132–133°.

0.1188, when dried at 110°, lost 0.0056 H_2O. $H_2O = 4.7$

0.1132 of anhydrous substance gave 0.3468 CO_2 and 0.1250 H_2O.

$C = 83.5$; $H = 12.2$

$C_{27}H_{46}O,H_2O$ requires $H_2O = 4.5$ per cent.

$C_{27}H_{46}O$ requires $C = 83.9$; $H = 11.9$ per cent.

This substance is thus seen to be a phytosterol, and a determination of its optical rotatory power gave the following result:

0.2722 of anhydrous substance, made up to 25 c.c. with chloroform, gave α_D — 0° 40′ in a 2 dcm. tube, whence α_D — 30.6°.

The acetyl derivative, when crystallized from acetic anhydride, separated in needles melting at 110°.

The alkaline liquid from which the above-described phytosterol had been extracted by means of ether was acidified and again extracted with ether, the ethereal liquid being dried and the solvent removed. A quantity of fatty acids in the form of a dark green mass was thus obtained. These acids were distilled under diminished pressure, and, by means of their lead salts, were separated into solid and liquid portions. The amount of solid acid obtained was 3 grammes. It distilled between 220 and 230°/$_{15mm}$., and, when recrystallized from ethyl acetate, melted at 64°.

0.1320 gave 0.3634 CO_2 and 0.1470 H_2O. C = 75.1; H = 12.4
$C_{16}H_{32}O_2$ requires C = 75.0; H = 12.5 per cent.

The solid acid thus appeared to consist of nearly pure palmitic acid.

The liquid acids distilled between 215 and 225°/$_{15mm}$. and amounted to 2.5 grammes. Determinations of the iodine and neutralization values gave the following results:

0.2276 absorbed 0.2308 iodine. Iodine value = 101.4
0.2034 neutralized 0.4025 KOH. Neutralization value = 197.9
$C_{18}H_{34}O_2$ requires Iodine value = 90.0; Neutralization value = 198.9

These results indicated that the liquid acids consisted chiefly of oleic acid, with a very small amount of an acid of a higher degree of unsaturation.

Ether, Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

These extracts were dark brown, resinous masses, and amounted to 24.7, 35.0, 47.0, and 150 grammes respectively.

The ether and chloroform extracts were examined by shaking their respective solutions successively with aqueous sodium carbonate and sodium hydroxide. Furthermore, all the above-mentioned extracts were heated with 5 per cent. sulphuric acid in aqueous

alcohol, and with a 10 per cent. solution of sodium hydroxide, but by none of these methods could any definite product be obtained from them.

Fusion of the Resin with Potassium Hydroxide.

A quantity (25 grammes) of the powdered resin was gradually introduced into 150 grammes of potassium hydroxide in a state of fusion, and the temperature of the mixture maintained at about 260° for some time. After cooling, the mass was dissolved in water, the solution acidified with sulphuric acid, and distilled in a current of steam. The distillate contained some volatile acid, which was converted into a barium salt, the latter amounting to 2.5 grammes. An examination of this salt showed the volatile acid to consist chiefly of a mixture of formic and butyric acids.

After the removal of the volatile acids, as above described, the liquid in the distillation flask was separated by filtration from a quantity of resinous material, and extracted with ether. The ethereal liquid was then shaken successively with a solution of sodium carbonate and a 10 per cent. solution of sodium hydroxide. The sodium carbonate liquid was acidified and extracted with ether, but on evaporating this ethereal liquid only a small amount of a tarry residue was obtained, the solution of which gave a green color with ferric chloride. The solution of sodium hydroxide removed nothing from the ethereal liquid, and on finally evaporating the latter a small amount of a dark colored, amorphous product was obtained, which possessed an exceedingly unpleasant odor.

Notwithstanding the very complete examination to which the roots of *Lasiosiphon Meissnerianus*, Endl., have been subjected, it will be seen that they have yielded but little of chemical interest. The chief constituent of the root is an amorphous resin, to which, as had previously been observed, its acrid properties are evidently due.

In conclusion, the author desires to express his indebtedness to Dr. F. B. Power for having suggested this research, and for the kind assistance he has afforded throughout the course of the work.