

Lantanin, the Active Principle of *Lantana camara* L. Part I.—Isolation and Preliminary Results on the Determination of its Constitution.

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Lantana camara is a very popular ornamental plant especially grown in hedges. It is not indigenous to South Africa but has been introduced from America. It is widely spread along the Natal coast. In the course of investigations by Steyn and Van der Walt (1941) into the disease occurring among dairy cattle near Durban, poisoning caused by this plant was suspected. The symptoms of poisoning by *Lantana camara* are very similar to those described by Quin (1933) in cases of poisoning with *Lippia rehmanni* and *Lippia pretoriensis*.

The toxicity of the plant obtained from the Durban area was duly investigated (Steyn and Van der Walt, 1941) and a sample of the dry leaves was submitted to the author for chemical investigation.

EXTRACTION.

From preliminary experiments the following general method of extraction has been adopted.

Five hundred (500) gm. of the dry powdered leaves of the plant in the flowering and seeding stages were extracted at room temperature with 1,000 ml. 96 per cent. alcohol. After filtration the alcohol solution was clarified with activated charcoal. A light-brown filtrate resulted. This filtrate was concentrated to about 500 ml. by evaporating the alcohol by means of a fan when spontaneous crystallization took place. The yield was approximately 0.3 per cent. per dry weight of leaves. After repeated re-crystallizations from 96 per cent. alcohol it melted at 276-280° C. with decomposition. The amount of this compound in different samples of plant investigated varied considerably, viz., from 0.31 to 0.68 per cent. per dry weight of powdered leaves.

The name suggested for this substance is "Lantanin".



Fig. 1.—Lantanin. M.P. 276-280°. ($\times 50$).

*MICRO-ANALYSIS OF LANTANIN.

	%C	%H	Mol. Wt. Rast.
Found	75.06	8.97	516
Calculated for $C_{31}H_{44}O_5$	74.96	8.93	497

Two (2) gm. of Lantanin dosed *per os* to a sheep which was then exposed to direct sunlight, caused photosensitization and a severe icterus similar to that observed by administering the plant.

PROPERTIES OF LANTANIN.

(1) Lantanin crystallises in prismatic needles from 96 per cent. alcohol. It is colourless, tasteless and odourless.

(2) It is insoluble in water but very soluble in ether, chloroform, carbon tetrachloride, benzene, pyridine, acetone, ethylacetate, methanol, petroleum ether, glacial acetic acid and acetic anhydride.

(3) It is insoluble in hot concentrated HCl.

(4) It is insoluble in hot dilute sulphuric acid but dissolves in concentrated sulphuric acid with an orange colour. On gentle heating the solution becomes red and afterwards carbonization occurs.

(5) It is insoluble in 25 per cent. nitric acid, but dissolves in 65 per cent. nitric acid with a yellow colour. A yellow precipitate forms on cooling.

* All the micro-analyses by Dr. Backeberg of the University of the Witwatersrand, Johannesburg, to whom I am very much indebted.

- (6) An alcoholic solution gives no colour reaction with ferric chloride.
- (7) Dilute alkaline potassium permanganate solution is decolourised, especially on warming.
- (8) Dissolved in carbon tetrachloride, Lantanin decolourises Bromine-water.
- (9) Tests for nitrogen were negative.
- (10) Ignited on a platinum disc, Lantanin burns with a very smoky flame leaving no residue.

OPTICAL ACTIVITY.

151.8 mg. Lantanin was dissolved in 8 ml. chloroform and the rotation, using a 10 cm. tube, determined

Rotation	= 1.72°
Blank	= 0.06°
∴ θ	= 1.66°
∴ $[\alpha]_D^{20}$	= $\frac{1.66 \times 1000 \times 8.}{151.8 \times 1}$
			= +80.74 (CHCl ₃).

No change of the rotation was observed when left overnight.

DETERMINATION OF THE OXYGEN FUNCTIONS OF LANTANIN.

I. *Lactone Grouping.*

Lantanin exhibits no acid action.

(i) To 115 mg. Lantanin, 10 ml. $\frac{N}{10}$ × 0.9852 was added and refluxed for 3 hours and the excess NaOH titrated.

ml. $\frac{N}{10}$ NaOH added	= 9.85
ml. $\frac{N}{10}$ NaOH back-titrated	= 9.75

It is therefore evident that Lantanin is not an acid nor an ester.

(ii) 50 mg. Lantanin was dissolved in 10 ml. 96 per cent. alcohol and then titrated at room temperature with $\frac{N}{10}$ alcoholic KOH using phenolphthalein as indicator.

ml. $\frac{N}{10}$ KOH used	= 1.04.
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Theoretical for one lactone group in 50 mg. Lantanin = 1.004 ml. $\frac{N}{10}$ KOH.

It is therefore evident that the Lantanin molecule contains a lactone group, accounting for two oxygen atoms.

Molecular weight.—From the titration value the molecular weight of the substance was calculated to be 480.

Theoretical for C₁₁H₄O: 497.

Relactonisation of Hydroxy-acid.—50 mg. Lantanin dissolved in 10 ml. alcohol and titrated with $\frac{N}{10}$ alcoholic KOH required
1.04 ml.

On addition of 5 ml. $\frac{N}{10}$ HCl the lactone was immediately reformed. From the solution Lantanin with M.P. 276—280° C. was recovered. Mixed melting point with pure Lantanin gave no depression.

It is therefore evident that the lactone is easily titrated at room temperature with alcoholic potash and that the hydroxy-acid is immediately relactonized with hydrochloric acid which is characteristic of γ -lactones.

Isolation of the potassium salt of the saponified lactone.—One (1) gm. of Lantanin was dissolved in excess alcoholic potash solution. The excess KOH was neutralized with hydrochloric acid and the mixture evaporated to dryness. The residue was extracted with ethyl acetate. On evaporation of the solvent 1 gm. of feathery crystals were obtained. After repeated crystallization from ethyl acetate it melted sharply at 229° C. with decomposition.

The potassium salt is insoluble in water; soluble in alcohol, methanol, chloroform, acetone, ether, benzene and ethyl acetate.

A sample of the potassium salt was digested with sulphuric acid and the potassium determined by precipitation as potassium cobaltinitrite and the potassium determined titrimetrically.

Analysis:—

	%K.
Found	6.59, 6.55
Calculated for $C_{31}H_{48}O_6K$	7.07

II. Ketone Group.

(a) *Preparation of semi-carbazone.*—0.2 gm. of Lantanin dissolved in 25 ml. absolute alcohol was refluxed with 0.2 gm. semi-carbazone hydrochloride and 0.2 gm. anhydrous sodium acetate dissolved in 1 ml. water. After $\frac{3}{4}$ hour \pm 0.2 gm. of a white crystalline material separated which upon repeated crystallization from absolute alcohol gave a M.P. 285° C., with decomposition.

Micro-analysis:—

	C%	H%	N%
Calculated for $C_{32}H_{47}O_6N_3$	69.59	8.55	7.59
Found	70.31	8.62	7.66

It is therefore evident that a ketone group exists in the Lantanin molecule and the above further confirms the empirical formula of Lantanin.

(b) *Preparation of the 2:4 dinitrophenylhydrazone.*—0.2 gm. Lantanin was dissolved in 30 ml. absolute alcohol and 0.2 gm. 2:4 dinitrophenylhydrazine dissolved in 5 ml. absolute alcohol was mixed and 2 ml. 25 per cent. HCl added. 0.2 gm. orange-coloured prismatic needles crystallized after 15 minutes.

After repeated recrystallization from absolute methanol, the crystals melted sharply at 268° C.

Micro-analysis :—

	C%	H%	N%
Found	66.72	7.43	8.74
Calculated $C_{37}H_{48}O_8N_4$	65.66	7.15	8.27

III. Hydroxy Groups.

(a) *Methylation of Hydroxy Groups in Lantanin*.—To 10 ml. of a cold saturated solution of Lantanin in methyl alcohol (containing approximately 0.3 gm. Lantanin), 2 ml. nitroso-n-methyl-urethane was added and then 10 ml. of a cold saturated solution of KOH in methyl alcohol added and gently mixed in an icebath.

The mixture was left overnight and then evaporated to dryness. After the addition of distilled water a white substance separated out which was filtered, washed and recrystallized from absolute alcohol. After repeated recrystallizations it melted at 208-213° C. and was negative for nitrogen.

Micro-analysis :—

	C%	H%
Found	75.95	9.07
Calculated for 2 hydroxy groups, viz., $C_{33}H_{44}O_8$	75.53	9.22

(b) *Preparation of the acetyl derivative*.—1.0 gm. Lantanin was refluxed for 3 hours with 3 ml. of acetic anhydride and 0.5 gm. anhydrous sodium acetate.

The mixture was then poured into ice-water when 0.9 gm. of a white substance separated out. It was filtered, washed and repeatedly crystallized from 96 per cent. alcohol, when it melted with decomposition at 246-250° C.

Refluxing 88 mg. of the acetyl derivative for 6 hours with 10 ml. $\frac{N}{10}$ alcoholic potash, 3.52 ml. $\frac{N}{10}$ KOH was used.

ml. $\frac{N}{10}$ KOH used for saponification of the lactone group in 88 mg. acetyl derivative = 1.77

∴ ml. $\frac{N}{10}$ KOH used for the saponification of the acetyl group in 88 mg. acetyl derivative = 1.75.

Theoretical value for 1 acetyl group = 1.68 ml.

This indicates that one hydroxy group was acetylated.

Micro-analysis :—

	C%	H%
Found	75.73	9.06
Calculated for one hydroxy group [$C_{33}H_{44}O_8(OCOCH_3) - H_2O$]	76.01	8.51

From the micro-analysis it would appear that acetylation took place with the loss of one molecule of water. This would explain the difference in the number of hydroxy groups established by means of methylation and acetylation.

ETHYLENIC DOUBLE BONDS.

Preliminary qualitative tests showed that Lantanin was unsaturated towards Bromine-water and Potassium permanganate.

Using 96 per cent. acetic acid as solvent and platinum dioxide as catalyst, 5 gm. Lantanin was hydrogenated until the hydrogen absorption was complete. A blank experiment using the same amount of PtO_2 as before was conducted and it was found that 495 ml. hydrogen had been absorbed at 21°C . and 644 mm. pressure.

Vol. H_2 absorbed (reduced to N.T.P.) = 389.5 ml.

Theoretical vol. for 5 gm. Lantanin for one double bond (at N.T.P.) = 225 ml.

Two double bonds of Lantanin have therefore been hydrogenated.

Separation of the tetra-hydro-derivative.—After hydrogenation the PtO_2 was filtered off. On standing the tetra-hydro-derivative crystallized in transparent needles. After further recrystallizations from 96 per cent. alcohol it sublimes at 237°C . and melts at 261.4°C . with decomposition.

Micro-analysis :—

	%C	%H
Found	73.74	9.60
Calculated $\text{C}_{31}\text{H}_{44}\text{O}_5$	74.31	9.65

As soon as more plant material is available it is hoped to conduct degradation of the Lantanin molecule by oxidation and dehydrogenation.

SUMMARY.

1. The photosensitising constituent of *Lantana camara L.* has been isolated and named *Lantanin*. The empirical formula is $\text{C}_{31}\text{H}_{44}\text{O}_5$.
2. The functions of the five oxygen atoms have been determined, viz., a lactone group, a keto group and two hydroxy groups.

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