Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 23, Numbers 1 and 2, March, 1948.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Lantadene A, the Active Principle of *Lantana camara L.* Part II.—Isolation of Lantadene B, and the Oxygen Functions of Lantadene A and Lantadene B.

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Lantana camara had been previously investigated from the viewpoint of commercial ethereal oils. Moudgoll *et al.* (1922) distilled an oil of a pleasant and persistent odour from the leaves of the plant. This oil contained 10-12 per cent l-α-phellandrene and about 80 per cent. of an unknown sesquiterpene. An amorphous compound $C_{15}H_{26}O$, which was not a terpene alcohol, could also be isolated from the oil.

Kafuku *et al.* (1935) conducted a more complete investigation of the ethereal oils of *Lantana camara.* They obtained a yellow aromtic oil in 0·053 per cent. yield when the whole plant was steam-distilled. This oil contained chiefly terpene compounds.

The anthocyanin colouring matter in the flowers of the plant was isolated by Lal (1936).

According to the literature no further investigations as regards the chemical composition of the plant have been undertaken.

In 1941 Steyn and van der Walt found that this plant caused losses among dairy cattle. The active principle was subsequently isolated by Louw (1943) and named " Lantanin ". According to Wehmer (1931), this name had already been given to an alkaloid isolated from *Lantana brasiliensis.* To avoid confusion and as the active principle of *Lantana camara* is in all probability a polyterpene derivative, the name " Lantadene A " is now suggested.

For the extraction of larger amounts of plant material an extractor as described by de Waal (1941) was employed. In this 15 Kg. powdered leaves could be extracted at a time, the extraction being made with hot alcohol instead of with cold alcohol as before. The extraction proceeded from four to five days when no more chlorophyll was dissolved.

On working up the crude Lantadene A which was obtained by this exhaustive extraction method, it was at once evident that there occurs another crystalline substance along with Lantadene A. This substance

could easily be separated from Lantadene A. Whereas Lantadene A crystallises from 96 per cent. alcohol in clusters of delicate needles, the new compound crystallises in more firm prisms. On repeated recrystallisation this compound melts at 295-300° C. with decomposition. According to the combustion analysis and molecular weight determinations, this substance has the empirical formula, $C_{33}H_{48}O_5$. The name suggested for this compound is " Lantadene B ".

By fractional crystallisation the separation of Lantadene B from Lantadene A can be effected almost completely, but a point is reached when the two substances crystallise together and further separation is then hindered. In this manner a product with M.P. 275-280° C. could be obtained after repeated recrystallisation from 96 per cent. alcohol. This product consists of Lantadene A with traces of Lantadene B.

The last traces of Lantadene B could not be removed by the use of solvents because the two substances have almost identical solubilities. Separation by way of the potassium compounds which were prepared by the hydrolysis of Lantadene A and B with alcoholic potash proved unsuccessful.

The total yield of Lantadene B was approximately 0·20 per cent. of the weight of the dry leaves.

The typical picture of acute photosensitisation and severe icterus, caused by dosing a sheep with 2·0 gms. of Lantadene A, could not be produced with Lantadene B administered in similar amount even when the animal was given a further dose of 2·0 gms. a few days later.

The Oxygen Functions of Lantadene A.

In a previous communication (Louw, 1943) the functions of the five oxygen atoms in Lantadene A were reported upon.

(a) Ketone Group.

Lantadene A exhibits halochromic characteristics e.g. when it is dissolved in concentrated sulphuric acid, a wine-red solution is obtained. It condenses easily with semicarbazidehydrochloride, 2,4-dinitrophenylhydrazine and hydroxylamine. Lantadene A gives negative silver-mirror and Fuschsine-tests, from which it may be concluded that the carbonyl group cannot be in an aldehyde function.

Lantadene A could not be condensed with p-nitroso—dimethylaniline to form an azomethine or anil, which indicates that the carbonyl group cannot be situated next to a methylene group.

Attempts to reduce the ketone group to a methylene group according to the method of Clemmensen was unsuccessful from which it may be inferred that the ketone group must occupy a strongly sheltered position.

There is no indication that this ketone group exhibits keto-enol isomerisation. In preparing the ketone-derivatives condensation occurred readily whether precaution against enolisation was taken or not.

(*b*) *Lactone Group.*

A δ-lactone group was found to be present in Lantadene A. The hydroxy-acid formed on hydrolysis of Lantadene A, could easily be relactonised by hydrochloric acid.

(*c*) *Hydroxyl Function.*

Tests for phenolic hydroxyl groups in Lantadene A are negative. It gives no colour reaction with ferric chloride and does not form an azo-dye when treated with a solution of a diazonium salt.

When Lantadene A was treated with acetic anhydride, the product was a mono-acetyl derivative; it is probable that loss of one molecule of water occurred during the process. At this stage there is uncertainty as to what happened during acetylation.

Attemps at preparing a urethane and benzoyl derivative failed.

According to the first communication, methylation of LantadeneA indicated the probable presence two hydroxyl groups. From later experiments the presence of at least one hydroxyl group in Lantadene A could be shown by methylation. It became evident that the methylated product with M.P. 208-213° C. which had been previously reported upon, was in all probability a mixture of methyl ethers because it was found that two homogeneous products with M.P. 125° C. and 225° C. could be obtained by methylation. Fractions with melting points ranging between 125° C. and 225° C. were observed. The product with M.P. 125° C. could be prepared in 10 per cent. yield and proved to be the methyl ether of Lantadene A.

The product with M.P. 225° C. was obtained from the reaction mixture in very small yield and was proved to be the methyl ether of Lantadene B. The other fractions are mixtures of the ethers of Lantadene A and B, which co-crystallise and are difficult to separate.

The difficulty of co-crystallisation of Lantadene A and B has been pointed out. In the preparation of the methyl ether of Lantadene A this difficulty was again encountered.

From combustion analysis and determination of the methoxyl groups in the methylated product of Lantadene A, the presence of one hydroxyl group was confirmed.

There exists uncertainty as regards the fifth oxygen atom. Although it may appear that the fifth oxygen atom may also be in an hydroxyl function, it is very probable that it is present in an ether binding and more likely in an ether bridge. No methoxyl or ethoxyl groups could be found in Lantadene A. Higher alkyloxyl groups could not be determined by the Zeisel method. An indication that the fifth oxygen cannot be in an ordinary ether binding is that Lantadene A-resin, $C_{17}H_{26}O_3$ (to be reported on later) in which the fifth oxygen atom must still be present, yields the hydro-carbon $C_{17}H_{16}$ on dehydrogenation. From this it is evident that a R^1OR^2 arrangement could not have existed, otherwise a loss of C atoms would have occurred.

Two ethylenic double bonds were shown to be present in Lantadene A. Lantadene A could not be condensed with maleic-anhydride from which it is evident that the two double bonds are not in a conjugated position to each other.

Oxygen Functions of Lantadene B.

The presence of a lactone group, a ketone group and at least one hydroxyl group was found in Lantadene B. The function of the fifth oxygen atom has not been determined.

EXPERIMENTAL.

Lantadene A-Methylether M.P. 125° C.

This product was prepared by methylation of Lantadene A. M.P. 275-280° C. and purified by fractional recrystallisation.

(1) *Analysis.**

3·529 mgm. substance ; 9·707 mgm. CO_2 ; 2·848 mgm. H_2O
Calculated $C_{32}H_{46}O_5$: C = 75·23% ; H = 9·08%
Found..... : C = 75·02% ; H = 9·03%

(2) *Methoxyl content of ether M.P. 125° C.*

Micro-methoxyl determinations were made according to the method of Pregl.

(*a*) 8·00 mgm. substance
Titration : 0·75 ml. $\frac{N}{10}$ × 1·0362 $Na_2S_2O_3$

(*b*) 7·05 mgm. substance
Titration 0·70 ml. $\frac{N}{10}$ × 1·0362 $Na_2S_2O_3$

Calculated for one—OCH_3 in $C_{32}H_{46}O_5$: 6·07% OCH_3
Found : (*a*) 5·023% OCH_3
(*b*) 5·319% OCH_3

Lantadene B.

(1) *Titration.*

Lantadene B is not an acid, but can be titrated with alcoholic potash in the cold (Lactone.)

0·4105 gm. Lantadene B (acetone) dissolved in 50 ml. 96 per cent. alcohol was titrated at room temperature with alcoholic potash using phenolphthalein as indicator.

ml. $\frac{N}{10}$ KOH used = 7·425.

$$\therefore \text{ Mol. weight (mono-lactone)} = \frac{10{\cdot}000 \times 0{\cdot}4105}{7{\cdot}425} = 552{\cdot}$$

* All micro-molecular weight, C, H and N determinations were made by Dr. O. G. Backeberg and Mr. J. L. C. Marais of the University of the Witwatersrand, to whom indebtedness is expressed.

(2) *Specific Rotation.*

Weight 82·8 mgm. Volume = 10 ml. $CHCl_3$
Tube = 1 dm. θ = 0·76°
$\therefore [\alpha]_D^{21} = +91{\cdot}8$ $(CHCl_3)$

(3) *Analysis.*

3·622 mgm. L.B. (acetone) : 10·079 mgm. CO_2; 2·965 mgm. H_2O
3·492 mgm. L.B. (acetone) : 9·693 mgm. CO_2; 2·819 mgm. H_2O

Calculated $C_{33}H_{48}O_5$	: C = 75·53%;	H = 9·22%
Found..... (i)	: C = 75·89%;	H = 9·16%
,, ... (ii)	: C = 75·71%;	H = 9·03%
Mol. weight	: Calculated $C_{33}H_{48}O_5$	: 524.
Found (titration)	:	: 552·

(4) *Oxygen Functions of Lantadene B.*

(*a*) *Ketone Group.*

Lantadene B condenses readily with (2,4-dinitro-phenyl hydrazine to form a hydrazone with M.P. 262-3° C.

Analysis.

Calculated $C_{39}H_{52}N_4O_8$	: C = 66·45%;	H = 7·437%;	N = 7·945%
Found.....	: C = 66·90%;	H = 7·48% ;	N = 8·33%
	: C = 67·04%;	H = 7·29% ;	N = 8·34%

(*b*) *Hydroxy Group.*

Methylether.

The methylether of Lantadene B was prepared by the use of nitroso-n-methylurethane. Here no difficulty with methylation fractions was encountered. Only one product with M.P. 225° C. could be isolated.

(i) *Analysis.*

Calculated $C_{34}H_{50}O_5$	: C = 75·78%;	H = 9·35%
Found.....	: C = 75·95%;	H = 9·31%
	: C = 76·07%;	H = 9·07%

(ii) *Micro-methoxyl determinations.*

Calculated for one methoxyl group in—

$C_{33}H_{18}O_5$: 5·76% OCH_3

Found.. : 5·36% OCH_3
5·42% OCH_3
5·93% OCH_3

(*c*) *Lactone Grouping.*

The ease with which Lantadene B can be titrated with alcoholic potash in the cold manifests the presence of a lactone group.

Treatment of Lantadene A with Maleic-anhydride.

(1) 3 gm. L.A. and 1·5 gm. anhydride were dissolved in 50 ml. glacial acetic acid, and the solution then refluxed for 24 hours. The solution was then cooled, water added, the precipitate collected, washed and dried and crystallised from alcohol. Only unchanged Lantadene A could be isolated.

(2) 0·5 gm. L.A. and 0·5 anhydride was dissolved in benzene and refluxed for 16 hours. The benzene was then distilled off and the residue taken up in 96 per cent. alcohol, water was added and the precipitate collected, washed, dried and crystallised from alcohol. Unchanged Lantadene A was obtained.

(3) 0·5 gm. L.A. and 0·5 gm. anhydride was dissolved in 20 ml. nitrobenzene and the solution refluxed for seven hours. After one hour the solution becomes yellow and then changes from orange-red to dark. After conclusion of the reaction, the solvent was removed by steam distillation and the residue taken up in alcohol. On concentrating the solution, unchanged Lantadene A crystallises. No crystalline addition product could be isolated.

Summary.

1. The active principle of *Lantana camara* L. has been renamed " Lantadene A ".

2. An inactive cogener of Lantadene A has been isolated and named " Lantadene B ".

3. The functions of the oxygen atoms in Lantadene A and B are discussed.

Acknowledgments.

Sincere thanks are due to Dr. J. I. Quin for testing Lantadene A and Lantadene B for their physiological activities.

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