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1923

# CASTELIN——A NEW GLUCOSIDE FROM CASTELA NICHOLSONI.

# AND

# CASTELAMARIN—A "BITTER PRINCIPLE" ASSOCIATED WITH CASTELIN.

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# CASTELIN.

Castela Nicholsoni (Torrey and Gray) belongs to the natural order of Simarubaceae. It is also known as Chapparo Armagosa and "Bitter Bush". The Simarubaceae have a wide geographical distribution. They are well represented in Mexico, Texas, Guiana, and also in Venezuela--in Central and South Americas. In the Far East we find them in China and Australia. In China there are no fewer than FIVE representatives of the genus Brucea: Power and Lees (Pharm. J., 1903, iv, 17, 183.)

Up to quite recently there seems to have been some difference of opinion as to the exact botanical classification of the herb in question. The above classification (Castela Nicholsoni), however, has been accepted by Nixon (J. Amer. Med. Assoc., 1916. 61. 946), and by Watson and Mc:Iver (J. Pharm. and Exp. Therap., 1913. 11. 331.)

The medicinal value of the Simarubaceae seems to have been known for some centuries. The natives of China, Mexico, and Texas have long since used decoctions of these herbs as remedies for dysentery, syphilis, rabies, yellow fever and malaria. Gradually the attention of the physicians in Texas was drawn to the curative properties of these decoctions (loc. cit.,) and Shepard and Lille (Prescriber. 1918. 96.)

In view of the many favorable reports as to the medical value of this and similar herbs, it seems rather strange that, up to the time of writing, nothing was known as to the chemical nature of the active principle. Putegnat is reported to have isolated a glucoside many years ago, but this was refuted (presumably by himself) (New Remedies 1883. XI. 102.)

The author proposes to assign the name CASTELIN to this glucoside and, in accordance with the usual glucosidic terminology, CASTELA-

GENIN, to the product of hydrolysis.

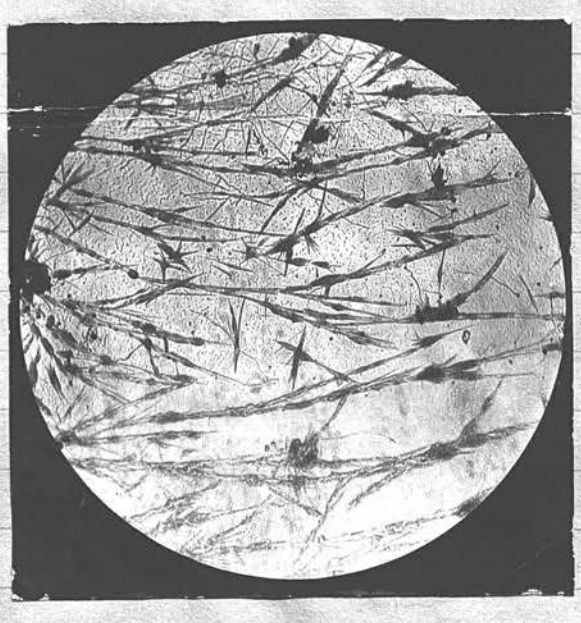
Associated with the glucoside there is a "bitter principle", which will be discussed later, under the heading of CASTELAMARIN. In passing it should be noted that the bark of Simaruba Amara of the natural order also contains a "bitter principle", the nature same. of which has been investigated by Gilling, who assigns the formula $C_{22}H_{30}O_9$ to it. (Pharm. J. 1908, iv, 26. 510.)

## EXPERIMENTAL.

The herb is extracted by continuous percolation with water or alcohol. On concentration of the aqueous or alcoholic extract, a non-glucosidic "bitter principle" separates ; and on further concentration of the mother liquor, the glucoside, castelin separates out. [For this information I am indebted to Prof: Barger---not having extracted the glucoside myself.]

## PROPERTIES OF CASTELIN.

The glucoside is purified by repeated re-crystallisations from water. It separates out in long white arborescent-like needles, shewn in this micro-photograph.



X 45 Diameters.

It's solubility increases considerably with increase of temperature. At room temperature it dissolves in 85 parts of water (by weight), and in 25 parts of water at 100°C. It is also soluble in ethyl alcohol and cold concentrated hydrochloric acid. It melts at 204-205°C, and gives a violet coloration with concentrated sulphuric acid. This deep violet color fades to a brownish tint after a few minutes. The anhydrous substance is very hygroscopic. On exposure to air for 60 minutes it takes up 8 per cent. of water. Special precautions have, therefore, to be taken in combusting the material.

0.2082 grs: anhydrous subst: gives 0.4181 grs: $CO_2$ and 0.1264 grs: water ($H_2O$).

Whence C = 54.8 and H = 6.8 per cent.

$C_{15}H_{22}O_8$ requires C = 54.6 and H = 6.7 per cent.

A number of combustions were done, another result being:-

0.2050 grs: gives 0.4110 grs: $CO_2$ and 0.1286 grs: $H_2O$.

Whence C = 54.7 and H = 6.9 per cent.

The glucoside crystallises with THREE MOLECULES of water of crystallisation.

0.1114 grs: on drying at 120°C until constant loses 0.0160 grs: water, which is 14.4 per cent.

$C_{15}H_{22}O_8.3H_2O$ requires 14.1 per cent water. A similar result was obtained by exposing the substance to a vacuum in a desiccator.

Owing to the limited number of solvents which could be used, and the small solubility of the castelin in these in the cold, the molecular weight had to be determined by the Boiling Point method. The Walker-Lumsden modification of the Beckmann apparatus was used.

1.2124 grs: of the anhydrous substance in 15 c.c. of ethyl alcohol gives a rise 0.035° which gives a Molecular Weight = 342.

$C_{15}H_{22}O_8$ requires 330.

Castelin is Dextro-rotatory. $\{[a]_D = +62.9°.\}$

## CASTELAGENIN

Hydrolysis of castelin:- The glucoside is readily hydrolysed by dilute acids and alkalis.(It is only very slowly hydrolysed by emulsin).A series of experiments on these lines shew that a 20 per cent. solution of hydrochloric acid,as hydrolytic agent, yields the best results.The yield is not very good.On an average the splitting product(castelagenin) was never more than 20 per cent. of the castelin hydrolysed.On one occasion only did it exceed this.

The castelin is gently boiled under a reflux with the 20 per cent acid.

2 grs: were hydrolysed in this way with 40 c.c. of the acid.The splitting product separated in large colorlees crystals.A further separation takes place on standing over-night.The total yield was 0.4142 grs:.The process of hydrolysis runs to completion in about two hours.

The castelagenin is re-crystallised from boiling glacial acetic acid.It is soluble in warm methyl and ethyl alcohols,but it is insoluble in acetone,ether and chloroform.It melts at 240-241°C. It gives no violet color with concentrated sulphuric acid,and is not nearly so bitter as castelin.

(a).0.1146 grs: gives 0.2708 grs: $CO_2$ and 0.0714 grs: $H_2O$.

Whence C = 64.4 and H = 6.9 per cent.

(b).0.1116 grs: gives 0.2642 grs: $CO_2$ and 0.0686 grs: $H_2O$.

Whence C = 64.6 and H = 6.9 per cent.

$C_9 H_{12} O_3$ requires C = 64.3 and H = 7.1 per cent.

0.3606 grs: of castelagenin in 42 c.c. alcohol gives an elevation of 0.07°.

Thus the Molecular Weight is 174.

$C_9 H_{12} O_3$ requires 168.

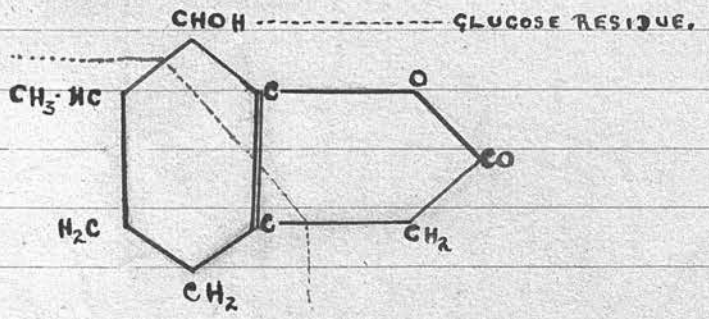
Since castelagenin is soluble in cold phenol, attempts have been made to verify this result by Freezing Point methods. The average result was 90. This is in accordance with the experience of Seel and Kelber (Ber. 1916, 49, 2364.) who, for aloin and some of it's oxidation products, found cryoscopically in phenol solution only half the correct molecular weights, which were obtained ebullioscopically in acetone solution and ethyl alcohol.

Castelagenin is Dextro-rotatory $\{[\alpha]_D = +59°\}$. It dissolves at once in cold sodium hydroxide, but only slowly in sodium carbonate in the cold. In warm sodium carbonate, however, it dissolves readily. It is evidently not a carboxylic acid or a phenol. It is not unreasonable to suggest that it is a substance which is LACTONIC in nature.

In a quantitative experiment 0.0556 grs: of the castelagenin was dissolved in 2.40 c.c. of N/10 NaOH and this required 0.64 c.c. of N/10 HCl for neutralisation. This means that the equivalent weight of the castelagenin is 317. This, obviously, is not compatible with the results cited above, but it is pointed out by Hans Meyer that the results obtained for the molecular weights of an aromatic lactone, by the method of titration, are not reliable. ("Analyse und Konstitutionsmittelung Organischer Verbindungen").

At one stage of his investigations, the author suspected that the substance was of a coumarin nature, but the high hydrogen content and the optical activity dispel of such an idea.

Of the three oxygen atoms, two are probably in the lactone group, and the third is in an hydroxyl group united to the glucose residue. It is hardly justifiable to speculate too much, but a formula of this nature would not be impossible.




On oxidation with nitric acid(30 per cent. at 150°C), castelagenin yields a minute quantity of a crystalline acid. This acid is moderately soluble in ether, and gives the FLUORESCEIN reaction. It appears as though the oxidation product is a di-carboxylic acid. Castelagenin yields a similar product when treated with potassium permanganate---both in alkaline and in acid solutions. As there was so small a quantity of the castelagenin available, the amount of the oxidation product was so minute in quantity that no definite conclusions could be drawn as to it's exact chemical constitution. For the present it can only be said that there is a strong suspicion that it is a substituted succinic acid. This might conceivably be formed from a substance with the formula postulated above.

The castelagenin was subjected to potash fusion, bromination, and methylation. In each case the products were carefully examined but nothing definite could be concluded from these investigations.

## The Nature Of The Sugar(s) Formed On Hydrolysis.

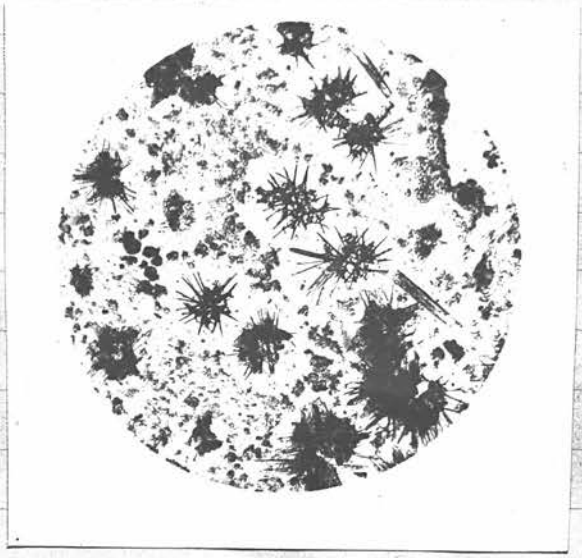
The mother liquors from the castelagenin were examined as to nature of the sugar(or sugars) which was formed on hydrolysis of the glucoside castelin. It was only after a careful study of the best

method of hydrolysis that a definite result could be obtained. The osazone was then readily prepared, and re-crystallised from methyl alcohol and pyridine. It melted at 206-209°C. This corresponds with the melting point of phenyl-glucosazone. The crystalline form of the osazone is also identical with that of the phenyl-glucosazone.

A quantitative experiment on these lines did not yield good results.

## CASTELAMARIN.

Professor Barger kindly presented the author with a few samples of the "bitter principle" associated with the glucoside castelin. These, however, were of varying degrees of purity, and the author tried various solvents in his search for a suitable crystallising medium. Petroleum ether, ether, acetone, glacial acetic and dilute acetic acids, ethyl acetate, alcohol and water were tried. After a prolonged series of experiments, it was found that 60 per cent. alcohol was the most suitable medium. From this the castelamarin crystallises out in aggregates of thin needles. (This microphotograph does not shew the substance in it's purest form).



X 50 Diameters.

From 0.5 grs: of the impure castelamarin 0.31 grs: was obtained. The yield, therefore, is a trifle over 60 per cent. Repeated attempts to get more from the mother liquors were without result. (The residue from the mother liquors is a resinous-like material, and is still being investigated.)

The castelamarin, after repeated re-crystallisations, was dried at 120° C, (for 3 hours) It melts at 265-267°C. It can also be purified by dissolving it in caustic soda (dilute), and re-precipitating it with dilute hydrochloric acid.

0.2710 grs: of the impure castelamarin were dissolved in 10 c.c. of water to which 3 drops of a 40 per cent. caustic soda solution were added. It was SLOWLY re-precipitated by a little hydrochloric acid, and yielded a crystalline product similar to that above. The precipitated substance was freed from sodium chloride and dried at 120°C for three hours.

The product was of a slightly yellowish tint, so the process was repeated a few times. Finally it treated with a little animal charcoal. The purified product so obtained weighed 0.22 grs: This corresponds to a yield of 82 per cent. It melts at 267-269°C.

The material available was not very much, so the author combined all the specimens of Castelamarin, divided the quantity thus obtained into two, and purified each half----the one by means of 60 per cent alcohol, and the other by means of caustic soda and hydrochloric acid. These two products were investigated side side, because it has been suggested that the product differs according as to whether it has been treated with alkali or not.

For the convenience of the readers the results have been arranged in tabular form.

| CASTELAMARIN NOT TREATED WITH ALKALI. | CASTELAMARIN TREATED WITH ALKALI. |
|---|---|
| (a). Crystals needle shaped | Crystals needle shaped with occasional leaflets. (?) |
| (b). M.P. 265-267°C | M.P. 267-269°C. |
| (c). No loss after being dried for 3 hours at 120°C. | No loss after being dried for 3 hours at 120°C. |
| (d). Excessively bitter and gives a deep blue color with conc: $H_2SO_4$. This fades to a brown tint after a few minutes. | This product behaves in a similar way. |
| (e). (i) 0.1476 gives 0.3426 $CO_2$ and 0.1060 $H_2O$. C = 63.3 and H = 8.0 per-cent. | 0.1420 gives 0.3315 $CO_2$ and 0.1040 $H_2O$. C = 63.7 and H = 8.1 per-cent. |
| (ii) 0.1503 gives 0.3495 $CO_2$ and 0.1112 $H_2O$. C = 63.4 and H = 8.2 per-cent. | 0.1392 gives 0.3256 $CO_2$ and 0.1000 $H_2O$. C = 63.8 and H = 7.8 per-cent. |

$C_9 H_{14} O_3$ requires C = 63.53 and H = 8.23 per cent.

The author must, at this stage, confess that he sees no very well marked difference between the two products. These results evidently do not confirm this suspicion.

The MOLECULAR WEIGHT determination of the product from the 60 per cent. alcohol yields teh following result:-

0.0208 grs: in 10 c.c. of glacial acetic acid (sp.g. 1.105) produces an elevation of 0.027°, which gives a molecular weight of 176.6

M.W. = 176.6.

The castelamarin which was treated with alkali shews that:-
0.032 grs: in 10 c.c. of this glacial acetic acid gives an elevation of 0.042°.

M.W. = 174.3.

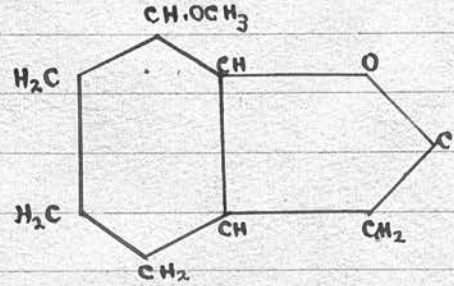
$C_9 H_{14} O_3$ requires 170.

A METHOXY DETERMINATION by Zeisel's method shewed that 0.2772 grs: of the castelamarin yields 0.3520 grs: silver iodide.

Whence $(CH_3O)$. = 16.8 per cent.

$C_8 H_{11} O_2(OCH_3)$ requires 18.2 per cent.

This would indicate that there is ONE methoxy group.
Attempts were made to split off the methoxy group by refluxing with hydrobromic acid, heating at 200°C with 15 per cent hydrochloric acid in a sealed tube for two hours, and also with alcoholic potash----but in no one of the above experiments were any definite results arrived at.
The author, growing suspicious that castelamarin might be related to castelagenin in some simple way, and naturally the idea of a methoxy derivative came in for a certain amount of consideration.




(may be considered a possible formula.)

The idea was to replace the methoxy by an hydroxyl group, and then to examine the product and to compare it with castelagenin. The evidence thusfar does not allow of such a speculation, but the author is not certain that it is incorrect.

HYDROLYSIS OF CASTELAMARIN.

1.48 grs:of the purified castelamarin were refluxed with 120c.c. of 2½per cent sulphuric acid.After 15 minutes most of it passed into solution.Within 45 minutes the substance had completely dissolved.The refluxing was continued for four hours.The acid solution was extracted three times with ether(80 c.c. at a time). The ether was distilled off and left a residue of .33 grs:.

On examination the residue was found to be of a resiny character. Scattered throughout the groundmass,however,there were a few crystalline leaflets.

Further extractions with chloroform yielded but very little of the above residue.Numerous solvents and mixtures of solvents were tried as crystallising media,but the results were not satisfactory.

From ethyl acetate,however,it was possible to get a few crystals but the yield was so small that it was practically negligible in amount.There was just sufficient for a few melting point determinations.It gives no coloration with concentrated sulphuric acid.It crystallises in long needles(as per photograph) and melts at 166-167°C.

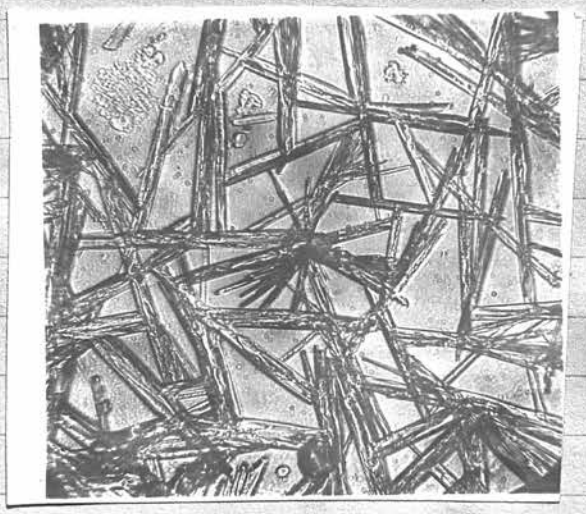
It is insoluble in cold benzene,but dissolves in alcohol,ethyl acetate,and warm chloroform.

The residue from the ethereal, chloroformic extractions was freed and from sulphuric acid by means of a saturated solution of barium hydroxide. The clear filtrate was taken down to a small bulk, but yielded nothing. Beyond the fact that the concentrated liquid possesses marked reducing properties, nothing further can be reported.

OXIDATION OF CASTELAMARIN.

Alkaline and acid potassium permanganate do not react as well as dilute nitric. .4 grs: of the castelamarin were oxidised with 10 acid c.c. of nitric acid. (1:3). This was well shaken when the castelamarin gradually dissolved in the cold. The mixture was then gently warmed for a few minutes on the water bath to facilitate the oxidation. The solution was then cooled, and neutralised and extracted three times with chloroform. It was found that three extractions were sufficient.

The chloroform extraction were worked up, but only a very small quantity of a crystalline substance was obtained, (.05 grs:). The product is freely soluble in chloroform and acetone. It is also soluble in other solvents, but warm rectified spirits is the most suitable crystallising medium. From this it crystallises as in the accompanying photograph.



X 150 Diameters.

To ensure the absence of inorganic material, some of it was heated on a platinum foil. It burned with an intensely smoky flame leaving no residue. It melts at 238-240° C. It is somewhat bitter to the taste, and gives no color reaction with sulphuric acid. The quality of the oxidation product appears to be satisfactory, but there was too little of the castelamarin to obtain enough of this for combustion analyses.

## SUMMARY.

(1). The active principle of Castela Nicholsoni is a glucoside--- Castelin. It melts at 204-205° C. It is fairly hygroscopic and has an empirical formula $C_{15}H_{22}O_8 . 3\ H_2O$.

(2). Castelin, on hydrolysis, yields castelagenin and glucose. Castelagenin is represented by the formula $C_9H_{12}O_3$. It is lactonic in nature, and it's probable structural formula is as suggested on p.6 of this thesis. It has a melting point of 240-241° C.

(3). Castelamarin is a "bitter principle" associated with this glucoside. It's formula is $C_9H_{14}O_3$, and it contains one methoxy group. It melts at 267-269° C.

## PHARMACOLOGICAL PROPERTIES OF CASTELAMARIN.

Having investigated the chemical nature of castelamarin, it was found that too little of the substance was left for any detailed pharmacological investigations. This rendered impossible the performance of a number of experiments which would otherwise have been carried out. No attempt was, therefore, made to determine the toxicity of castelamarin. The experiments were confined to it's action on isolated tissues, and to it's effects when given intravenously. The latter also gave an indication as to it's toxicity.

## EXPERIMENTAL.

.5 grs: of castelamarin was dissolved in 100c.c. Ringer containing 12 drops of a 30 per cent. solution of caustic soda. A solution of "mammalian" Ringer, containing the same amount of caustic soda was used as control.

### A. Effects on Isolated Tissues.

### (1). Action on Excised Uterus and Intestine.

The non-pregnant rabbit and the non-pregnant cat were used for these experiments. The animal was killed by a blow on the head and the uterus and a part of the intestine were quickly removed and placed in warmed oxygenated Lock's solution.

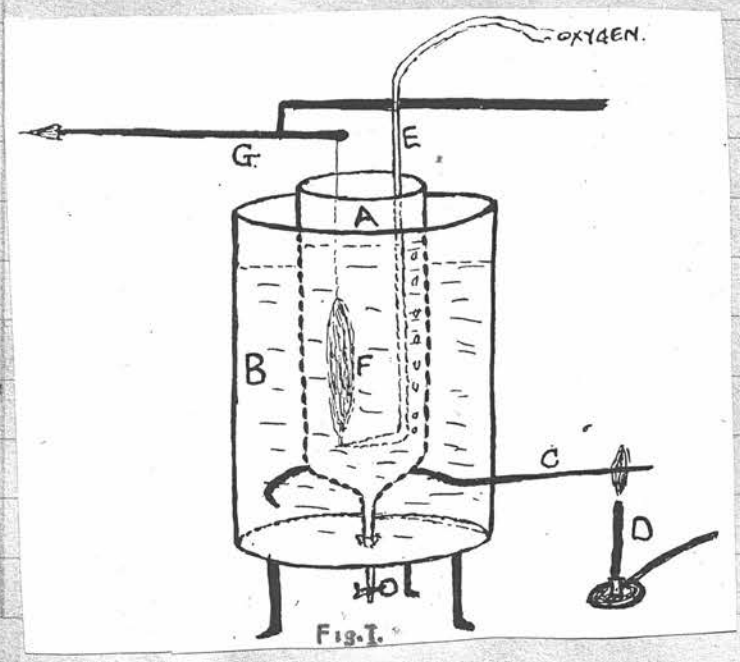


Fig.1.

The uterus or a piece of intestine about an 1" long was then suspended in Lock's solution at 37°C in an apparatus similar to that described by Dale and Laidlaw (J.Pharm.Expt.Ther., 1912, IV, 75.), as shewn in Fig.1.

The Lock's solution is placed in the inner vessel A,and is oxygenated by a constant current of oxygen through the tube E. The water jacket is maintained at a temperature of 37°C by heat conducted along the copper rod C from the Bunsen burner D.A holds 200 c.c.of fluid,so the final concentration of any solution added could easily be determined.When the movements of the uterus or the intestine became regular,measured amounts of a warmed .5 per cent solution were added.(A Lock's solution of castelamarin).In these and subsequent experiments ,control experiments were performed to eliminate any action of the caustic soda.
Strengths of solutions of castelamarin up to 1 in 500 were used, but had no apparent effect on the isolated intestine.On the rabbit's uterus the results were again negative,but on the uterus of a non-pregnant cat,a solution of castelamarin of 1in1000 effected a slight, almost neglible, increase of tone,which did not increase on bringing the strength of the experimental solution up to 1 in 500. (Figs: 2 and 3).

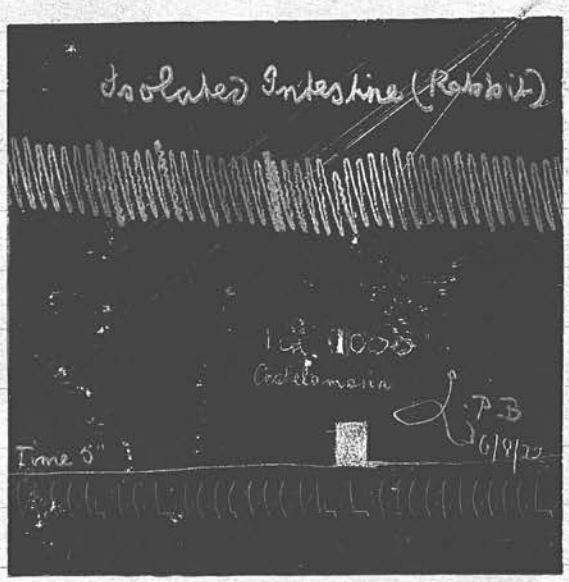



Fig.2.

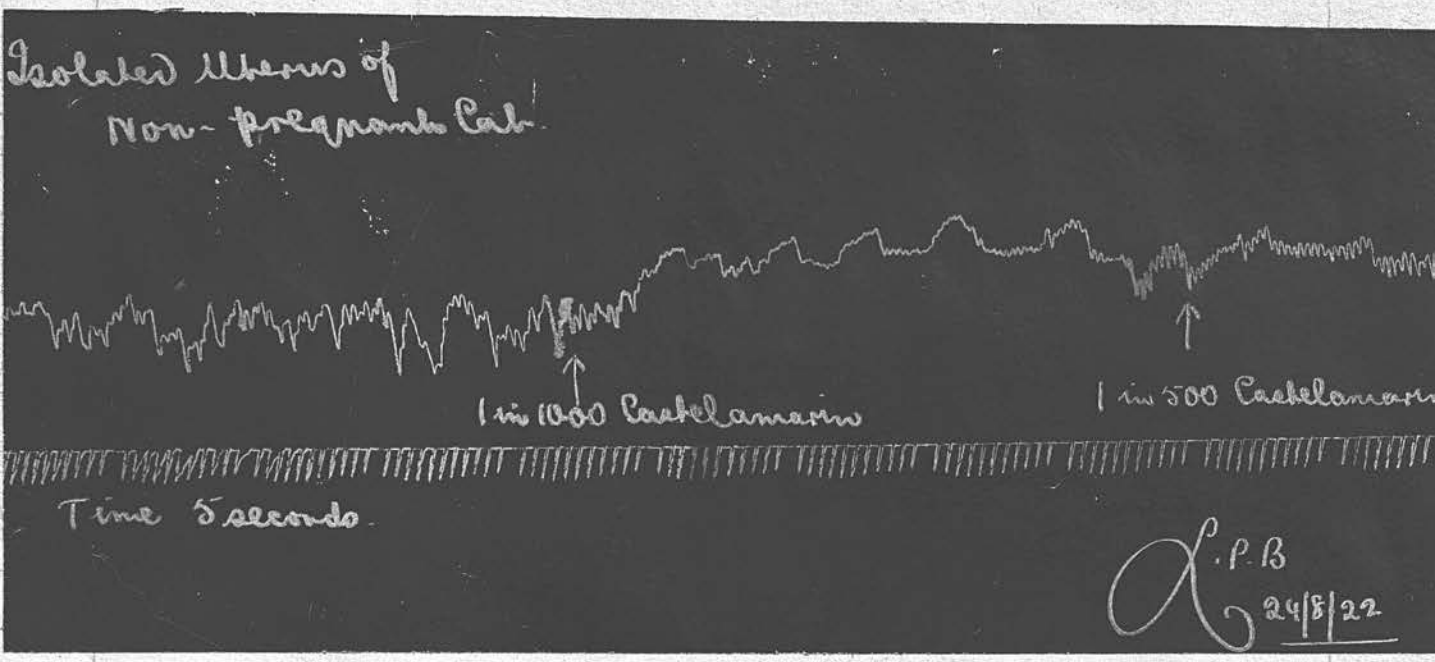



Fig.3.

(2).Effects on the Isolated Mammalian Heart.

The rabbit's and the cat's heart were used.The animal was killed as before,Lock's solution was perfused through the coronary arteries by the method of Largendorff.The apparatus used was that of J.A.Gunn.(J.Physiol.XLVI,506,1913.)The air round the heart was kept warm by the method of Cushny and Gunn.(J.Pharm.Expt.Ther.,V, 1,1913.).The apex of the heart was fixed to prevent swaying,and small hooks were attached to the auricular appendix and the ventricle,as described by Sherrington.(Mammalian Physiology p.10.1919) The flow through the coronary arteries was recorded by a siphon outflow recorder:—each 3 c.c. of the fluid being measured.The results of these experiments are shewn in Fig.4.(The auricular record is not shewn).

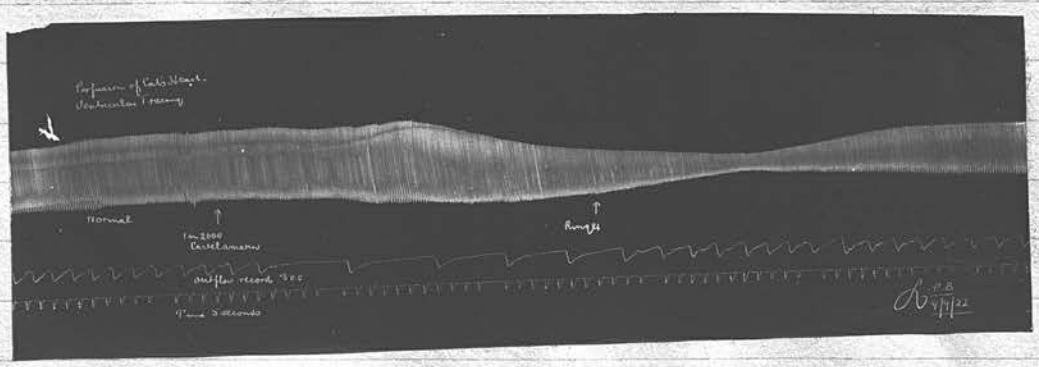



Fig.4.

The most striking effect of a perfusion with a 1/in 2000 solution of castelamarin was the constriction of the coronary vessels.This was indicated by the great retardation of the coronary outflow. Lock's solution perfused at the rate of 3.c.c.in 7.5 seconds,but after the castelamarin was perfused for a few seconds the rate fell to 3 c.c.in 32.5 seconds.At first the heart beat increased slightly in extent,but afterwards became smaller.The rate was unaltered. When the experimental solution was substituted by Lock's solution the heart recovered it's normal beat,and the coronary arteries also returned to the previous rate.It is noteworthy that the first effect,both on turning on and turning off the experimental solution,is the action on the coronary vessels.This would suggest that the heart muscle effects are secondary to the effects on the coronary circulation:i.e.castelamarin constricts the coronary vessels and the heart muscle is not sufficiently well supplied with oxygenated Lock's solution and the extent of systole diminishes:,or again perfusing the original Lock's solution,the vessels recover their previous calibre,and the heart muscle once more receives it's normal amount of nutrient fluid and returns to normal.

**B.Effects On Intact Animals.**

**Heart in Situ and Blood Pressure.**

The experiment,of which a tracing is shewn,was typical.A cat weighing 2.5 kilos.was anaesthetised with paraldehyde(1.5 c.c. per kilo.weight of cat).Canulae were inserted into the trachea, carotid artery and jugular vein in the usual way.The tracheal canula was connected to an artificial respiration pump and the chest opened in the middle line.The pericardium was opened and stitched to the sides of the chest wall.The movements of the ventricle were recorded by Cushny's myocardiograph.(Heart Vol.11.1.)

The blood pressure tracing, the injection, and the time were also recorded as shewn. Injections up to 40 mgrs: per kilo. of cat were made, and in some cases resulted in a small rise in the blood pressure (a few mm.)---but never more than the rise occasioned by the injection of the same amount of the control solution. It was, therefore, concluded that castelamarin has no appreciable effect on the heart and the blood pressure.

In one experiment 100 mgrs: per kilo. were given in divided doses without any more effect than that shewn. This indicates that castelamarin has a low toxicity when given intravenously.

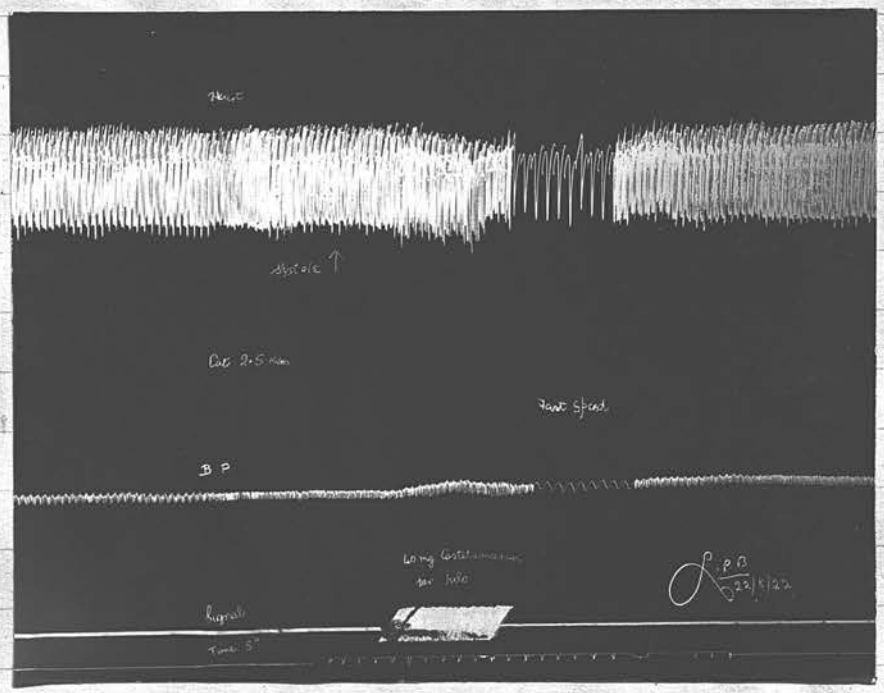



Fig.5.

## CONCLUSIONS.

These experiments, as stated before, are necessarily incomplete. They are, however, sufficient to lead us to state that castelamarin is a substance of low toxicity and of, at most, only slight pharmacological activity. Should a further supply of material be available at a later date, the work will be continued. There can be no

doubt that (~~that~~) the substance is effective in the treatment of amoebic dysentery: so it would be interesting to study the action of a solution of castelin and castelamarin on amoebae. For this purpose amoebae are being specially cultured.

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In conclusion the author wishes to emphasize his indebtedness to Professor George Barger F.R.S., of the Medical Chemistry Department, Edinburgh University, at whose suggestion and under whose supervision most of this work was carried out.

The author also takes this opportunity of thanking Professor J.W.C.Gunn, of the University of Capetown, for allowing him the use of the Pharmacological Laboratories, and for suggesting the lines on which to work, in order to put the very limited amount of material to the most profitable use.

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