

CHEMICAL COMPOSITION OF *CALOTROPIS GIGANTEA*

Part V. Further Examination of the Latex and Root Bark

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IN Part I of this series¹ a detailed examination of the wax and resin components of the latex of *Calotropis gigantea* was reported. The coagulum obtained by the addition of alcohol to the latex could be separated into two major fractions. The fraction soluble in ether and alcohol consisted of a resinol mixture from which two new resinols, α - and β -calotropeols and β -amyrin could be isolated and characterised. The alcohol and ether-insoluble portion of the coagulum was not examined in detail. The aqueous alcoholic filtrate from the coagulum, when extracted with chloroform, yielded a fraction whose properties and reactions indicated that it contained cardiac poisons. This too was not examined further. From the aqueous alcoholic mother-liquor left over, calcium oxalate was slowly deposited on long standing.

An attempt has now been made to complete the examination of the latex reported in Part I. The non-resinol portion of the coagulum has been found to consist mainly of caoutchouc. A quantitative estimation shows that caoutchouc forms 1.3% of the latex. A small amount of inorganic matter consisting mainly of calcium, oxalate, sulphide and phosphate is also present in the coagulum. The proportion of this is greater if larger volumes of alcohol are used for the coagulation of the latex or if the coagulum and the aqueous alcoholic portion are allowed to stand together for longer periods before filtration. The inorganic matter gets slowly precipitated after the addition of alcohol.

In the estimation of caoutchouc referred to above, acetone is employed for the removal of the more soluble components from the coagulum. This solvent is found to be more efficient than ether and alcohol for separating the resinols completely from the accompanying caoutchouc and mineral matter. When examined by the acetylation and benzylation methods as already described just the same components are obtained from this resinol fraction in a better yield. During the course of this work a number of

samples of the resinol mixture have been examined in detail. It seems to be definite that α - and β -calotropeols exist in the latex as such and are not produced by the reagents used for the separation. Even after a single crystallisation of the crude resinol mixture from rectified spirit a product is obtained which analyses essentially for the formula $C_{30}H_{50}O$ though small quantities of substances with the formula $C_{30}H_{50}O_2$ are not excluded. In another communication³ it has been shown that resinols with the latter formula which occur in the plant are also stable to the action of ordinary reagents. Further it has now been found that by repeated fractional crystallisation of the resinol mixture from the latex it is possible to obtain a small amount of α -calotropeol which is definitely pure.

For the isolation of cardiac poison from the aqueous alcoholic filtrate, observance of certain precautions has been found to be quite necessary for ensuring purity and yield. The substance is unstable to heat particularly in the presence of air and water and heating has to be avoided as far as possible. The resulting product has been carefully examined for the presence of more than one component, as in the work on the heart poisons of *Calotropis procera* by Hesse²; but only one entity is found to be present. The properties of this substance which contains nitrogen and sulphur and is poisonous to fish, resemble those of uscharin (isolated by Hesse) qualitatively but the two exhibit marked differences in details. It appears to be new and is therefore tentatively given the name 'gigantin'.

Properties	Uscharin	Gigantin
1. Crystal form ..	Fine needles	Small rectangular plates
2. Decomposition point ..	265°	243°
3. Optical Rotation $[\alpha]_D^{30}$..	29.0°	29.6°
4. Elements ..	C, 63.4; H, 7.0; N, 2.4 and S, 5.5%	C, 56.2; H, 7.1; N, 1.4 and S, 4.2%
5. Legal test ..	Positive	Positive
6. Hydrolysis with sulphuric acid	Uscharidin	Decomposition products

An examination of the root and stem barks of *Calotropis gigantea* has also been carried out with a view to see if they contain compounds of the cardiac poison group. The dry material is deprived of its wax components by extraction with petroleum ether and is then extracted with chloroform to get the cardiac poison. Here again it has been found that if continuous extraction using boiling solvent is employed the cardiac poison is destroyed as a result of the prolonged heating of the extract in the receiver, while if cold percolation is used and the solvent removed by distillation *in vacuo*

the residue gives definite tests for the presence of heart poisons. The stem bark is a very poor source; the root bark is much better though the product is very difficult to purify. Undoubtedly the latex is the best source.

There are two varieties of *Calotropis gigantea* which differ in the colour of the flowers. The purple coloured variety is more common and the white one is somewhat rare. The latter is considered to be more medicinal and more toxic than the former. In the course of the present studies the root bark of the white flowered variety has been found to contain much higher proportion ($1\frac{1}{2}$ to 2 times) of resinols and also of the cardiac poison.

EXPERIMENTAL

Estimation of caoutchouc.—A weighed amount of the dry coagulum (15 g.) was extracted thrice with boiling acetone (50 c.c. each time). This removed all the resinol portion. The residue was then boiled with a considerable excess of carbon tetrachloride or chloroform (150 c.c.). When no more solid went into solution, the supernatant solution was carefully filtered off from the small amount of inorganic residue. After washing the filter with a little more solvent the combined filtrate was distilled to recover most of the solvent. The residue was heated on a water-bath to remove the remaining solvent, dried in a steam oven, cooled and weighed (yield, 1.3% calculated on the latex). This substance, when dissolved in carbon tetrachloride gave a solution which was quite viscous and resembled commercial rubber solutions. The residue obtained after the removal of the solvent was flexible and elastic, and could be vulcanised by the usual methods employing sulphur and an accelerator.

Resinols in the coagulum.—The hot acetone extract was allowed to cool when a colourless solid was obtained. After the solvent was removed by distillation, a further quantity of the solid, which was slightly coloured, was obtained. Both the fractions gave colour reactions characteristic of resinols. So they were combined and saponified in benzene solution by boiling with 7% alcoholic potash. After removal of the solvents and addition of water to the concentrate, the unsaponifiable matter was extracted with ether, the ether solution dried over calcium chloride, and the solvent removed by distillation. A portion of the residue was crystallised from rectified spirit. A colourless solid was obtained in the form of flakes and it gave the following values when analysed for carbon and hydrogen: (Found: C, 83.8; H, 11.4; $C_{30}H_{50}O$ requires C, 84.5; H, 11.7% and $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.3%.)

The bulk of the unsaponifiable portion (m.p. 110–50°) of the coagulum (33 g.) was crystallised from benzene-alcohol (1:3) mixture fifteen times

when the melting point went up to 205–10°. Four further crystallisations from alcohol effected further rise in the melting point to 220–21° and this did not change on further crystallisation. The product (0.2 g.) was quite crystalline (transparent rods and narrow rectangular plates) and was identical with α -calotropeol obtained by the acetylation method in melting point and crystal structure. This sample was acetylated using acetic anhydride and sodium acetate when an acetate melting at 251–52° was obtained and this was identical with α -calotropeol acetate. Saponification of the acetate by boiling with 7% alcoholic potash yielded the original resinol again. On treatment with excess of bromine α -calotropeol acetate formed a bromo-derivative melting at 211–12°.

Isolation of gigantín.—The fresh latex was treated with an equal volume of alcohol and the mixture vigorously stirred. The coagulum that separated from the aqueous alcoholic solution was filtered after standing for an hour. The filtrate was concentrated *in vacuo* to remove alcohol as far as possible and the aqueous residue was repeatedly extracted with chloroform. The combined chloroform extract was washed with a little water, dried over calcium chloride and then evaporated under reduced pressure employing a very small stream of carbon dioxide to ensure smooth boiling. The residue was taken up in a small volume of chloroform and diluted with petroleum ether, added in small quantities at a time, the mixture being shaken after each addition. The clear solution was decanted off from the sticky residue which was obtained at each addition before adding the next portion of petroleum ether. When the precipitate obtained was no longer sticky but granular it was diluted with a large excess of petroleum ether and the solid filtered. Crude gigantín was thus obtained in a yield of 1% of the weight of the latex.

The purification was effected by dissolving the solid again in the minimum amount of chloroform and adding petroleum ether. After several repetitions of this process pure gigantín was obtained as small rectangular plates decomposing at about 243°. It gave the following values on analysis: C, 56.2; H, 7.1; N, 1.4; S, 4.2%. It had a specific rotation of +29.6° at 30°, in chloroform. Though nitrogen was one of the component elements it could not be detected qualitatively by Lassaigne's test evidently because of its low percentage but the presence of sulphur was easily indicated. Further an alcoholic solution gave a red colour with alkaline sodium nitroprusside (positive legal test). It dissolved in concentrated hydrochloric acid producing a greenish blue solution. With the Liebermann-Burchard reagent a green solution was obtained and the colour of the solution changed

to red after a day. The substance tasted bitter and left a tingling sensation on the tongue and it was toxic to fresh-water fish. When an attempt was made to hydrolyse it with N/2 methyl alcoholic sulphuric acid only an intractable product was obtained. Under similar conditions uscharin has been reported to give rise to uscharidin (Hesse, *loc. cit.*).

EXAMINATION OF THE ROOT BARK FOR CARDIAC POISONS

The dried bark was exhausted with petroleum ether in a continuous extractor to remove wax and the remaining material air-dried. It was subsequently percolated with cold chloroform and the percolate evaporated under reduced pressure using a small stream of carbon dioxide. The residue was treated further just as in the experiment with the aqueous alcoholic filtrate of the latex; it was very difficult to purify. The final product, obtained in very poor yield, was crystalline-looking and colourless and decomposed at about 210°. Due to dearth of material further purification could not be effected. The product, however, was toxic and gave all reactions of gigantol (yield, 0.1 g. from 1.5 kg. of the dry root bark).

SUMMARY

The latex of *Calotropis gigantea* contains caoutchouc to a small extent, 1.3%. The resinols are best separated from the coagulum by means of acetone. The mixture consists essentially of resinols having the formula $C_{30}H_{50}O$ and by repeated fractional crystallisation a very small yield of α -calotropeol could be obtained. The toxic principle of the latex, gigantol, contains nitrogen and sulphur and resembles uscharin in several respects, but differs from it in composition and in its behaviour on hydrolysis.

REFERENCES

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2. Hesse *et al.* .. *Ann.*, 1939, **537**, 67.
3. Balakrishna, Murti and Seshadri .. *Proc. Ind. Acad. Sci.*, A, 1945, **22**, 138.