CHEMICAL COMPOSITION OF *CALOTROPIS GIGANTEA*

Part VI. Flowers. A Comparison of the Composition of the Various Parts of the Plant

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THE flowers are ivory white outside and purple inside and are found all through the year. They contain a considerable amount of latex. A small quantity of the flowers was collected and examined with a view to see if they contain any special components and in a general way to effect comparison between these and the other parts of the plant. They could also be taken as representative flowers of the natural order Asclepiadaceæ.

The waxy matter of the flowers was extracted by means of ligroin. By careful fractionation it could be separated into two main fractions, one of which contained resinols and sterols as esters and the other consisted mainly of aliphatic components. Detailed analysis of the former revealed the presence of α- and β-calotropeols along with β-amyrin. A small amount of sterol could also be obtained. These were present as esters of volatile as well as non-volatile fatty acids. The aliphatic waxy matter seemed to consist almost entirely of esters derived from C_{34} alcohol and near homologues and acids of C_{28} and C_{30} dimensions. There were only small amounts of free acids.

The subsequent extraction with alcohol of the fat-free flowers yielded only mineral salts such as citrates and chlorides of sodium and potassium.

EXPERIMENTAL

The flowers of *Calotropis gigantea* were collected fresh in the month of September, the stalks were carefully removed and the remaining portions dried in the sun. The dry flower powder (1·0 kg.) was extracted in a continuous extractor with ligroin and alcohol in succession.

Ligroin extract:—The solvent was removed by distillation and the residue (15·0 g.) was dried in the steam oven. It was yellow in colour and waxy to the touch. For purposes of fractionation it was boiled with alcohol (400 c.c.) twice, the clear supernatant liquor was decanted off at about 60°

after each extraction and the solutions were mixed together. The sticky alcohol-insoluble portion (A) (6·0 g.) melted at about 50° and appeared to be a mixture of esters of resinols with solid fatty acids. The alcoholic solution (B) was concentrated to three-fourths of its bulk by distillation and was allowed to stand overnight. A pale yellow solid melting indefinitely between 120° and 150° separated out and on examination was found to be a mixture of aliphatic compounds and resinol derivatives. The above conclusion regarding the general nature of the components was arrived at after examining the solid with the Liebermann-Burchard and Salkowski reagents. In the former test a very turbid pink solution was produced and in the latter reaction a clean white solid slowly began to separate out indicating the presence of aliphatic wax. To effect the separation of these two types of components, the solid was again dissolved in alcohol and the solution allowed to cool slowly. The fraction that separated out melted at 80–120° and it was further purified by crystallisation from ethyl acetate. Finally after one more crystallisation from a mixture of acetone and ether (1 : 1) a resinol-free substance (B_1) melting at 88° was obtained. The original alcoholic mother-liquor of (B) obtained from the mixed components and the subsequent mother-liquors collected from (B_1), on further examination, yielded esters of resinols (B_2) melting indefinitely at about 200°. Its low yield did not permit of its individual study and hence (B_2) was added on to (A) and studied along with it.

Fraction ($A+B_2$) (*Resinols and Sterols*):—The total solid (10 g.) was dissolved in benzene (200 c.c.), N/2 alcoholic potash (500 c.c.) added and the contents were boiled under reflux for 15 hours. Then the major bulk of the solvents was removed and the concentrate was transferred to an open basin and mixed with pumice stone. The contents were rapidly dried and the dry mass was extracted in a Soxhlet extractor with dry acetone. The solvent was removed and the residue was dissolved in ether (500 c.c.). The ether solution was thoroughly washed with water in order to render it free of alkali and soap. Then the solvent was completely removed by distillation and the residue (6·0 g.) was dissolved in boiling alcohol (200 c.c.). On concentrating the solution to half its bulk a crystalline substance (C) melting at 165–75° separated out. Further concentration yielded some more of the above solid and both the fractions were mixed together. It produced the usual pink colour with the Liebermann-Burchard reagent and an yellow colour with the Salkowski reagent. The mother-liquors on still further concentration yielded fraction (D).

Fraction (*C*) (*Resinols*):—The fraction (3·8 g.) was dissolved in acetic anhydride (10 c.c.), pyridine (5 c.c.) added and the contents were boiled under

reflux for 3½ hours. They were then largely diluted with water, allowed to stand for some hours and the resulting solid was filtered. It was dissolved in boiling acetone (200 c.c.) and set aside for a day. A crystalline (indefinite) substance melting at 210° separated out. Two more crystallisations raised the melting point to 248° and the substance was obtained in the form of hexagonal plates. It gave all the colour reactions characteristic of calotropeols and their derivatives. No depression was noted in its mixed melting point with the acetate of α-calotropeol and hence its identity was established. From the mother-liquors on careful manipulation very small quantities of the acetates of β-calotropeol and β-amyrin were obtained.

Fraction (D) (Isolation of Phytosterol):—Its alcoholic solution was concentrated in stages and the resulting fractions were tested with the Liebermann-Burchard reagent at every stage. Such of those which produced only resinol colour reactions were rejected, the more soluble fractions which gave correct sterol colour reactions (display of colours with Liebermann-Burchard reagent) being collected separately. The fraction (1·5 g.) thus obtained was acetylated by boiling with acetic anhydride in presence of pyridine and the resulting product was ether extracted. The residue obtained after removing the solvent was subjected to repeated crystallisation from ethyl acetate and the top fractions were rejected. This precaution was found necessary in view of the fact that the resinols could not be definitely tested for in the presence of sterols and that there was possibility of the sterols being contaminated with them in the top fractions. From the tail fractions, after crystallisation from alcohol, a sharp melting acetate, m.p. 129°, was obtained in the form of colourless needles. It gave the correct reaction of sterols. The acetate (0·2 g.) was saponified by boiling with alcoholic potash; the free sterol thus obtained melted at 132°. (Found: C, 83·9; H, 12·8; $C_{28}H_{48}O$ requires C, 84·0; H, 12·0%.) Probably it is a sitosterol.

The Fatty Acids:—After extracting the unsaponifiables, the soap was acidified with sulphuric acid and steam distilled. The characteristic smell of isovaleric acid was noticed in the distillate and the presence of acetic acid was detected by testing with lanthanum nitrate. The non-volatile acids were then extracted with ether and the ether solution was dried. On removing the solvent completely by distillation, the acid residue (3·0 g.) melted indefinitely between 40° and 50°. One crystallisation from alcohol raised the melting point to about 67° and the mixture had a mean molecular weight of 388·6. It consisted, therefore, of solid acids with a mean chain length of 26 C atoms.

Fraction (B_1) (*Wax*):—This was sparingly soluble both in ethyl and methyl alcohols even in the hot, moderately soluble in ether and readily in benzene, petroleum ether and chloroform. It had an acid value of 20·0 indicating that most of it was made up of neutral compounds. To effect saponification, it (3·0 g.) was dissolved in benzene (200 c.c.), 7% alcoholic potash (100 c.c.) was added and the contents were boiled under reflux for 15 hours. Then an alcoholic solution (200 c.c.) of calcium chloride (12 g.) was added and the boiling was continued for two more hours. The solvents were distilled off to half the volume and the resulting solid consisting of calcium soaps and unsaponifiable matter was obtained by filtration. It was extracted thrice with dry acetone to dissolve the unsaponifiable matter and the acetone solutions were combined. The solid (1·2 g.) obtained after removing the solvent was crystallised from chloroform. It melted at 89° and appeared as rhombs under the microscope. Its acetate was prepared by boiling it (1·0 g.) with acetic anhydride (5 c.c.) in presence of pyridine (5 c.c.) for 3½ hours. The resulting mixture was largely diluted with water, cooled in ice and then ether extracted. On concentrating the ether solution a crop of crystals melting at 73–74° was obtained. (Found: C, 80·3; H, 12·9; $C_{36}H_{72}O_2$ requires C, 80·6; H, 13·4%.) The alcohol mixture was considered to have an average chain length of $C_{33\cdot5}$ and seemed to be a mixture of C_{32} and C_{34} alcohols mostly. The calcium soap left after the extraction with acetone was decomposed by boiling with dilute sulphuric acid. The liberated acid (1·3 g.) was crystallised from benzene-alcohol mixture and finally from chloroform. It melted at 87° and had a mean molecular weight of 448·8 and may therefore be a mixture of C_{28} and C_{30} acids.

Alcoholic Extract of the Flowers:—On concentrating the alcoholic extract to half its bulk (400 c.c.) and cooling in ice for some days a crystalline solid mixed with some resin settled down. It was purified by digestion with alcohol and the resin-free residue on examination was found to be a mixture of chlorides and citrates of sodium and potassium. It thus resembled the mineral matter obtained from the stem-bark.

Comparison of the Composition of Different Parts of *Calotropis gigantea*

A comparative statement of the composition of the various parts of *Calotropis gigantea* is presented in Table I.

There is marked difference in the occurrence of the aliphatic components in the different parts of the plant.[1] The stem bark contains the greatest percentage of aliphatic wax consisting mostly of a mixture of the free acids of C_{30}–C_{32} dimensions. The remaining portion of it is found to be made

TABLE I

	Stem bark	Latex	Root bark	Flowers
Aliphatic wax components—				
Free Acids ..	Present	Nil	Nil	Nil
Esters ..	Negligible	Nil	Nil	Present
Hydrocarbons ..	Present	Nil	Nil	Nil
Acids derived from resinol esters—				
Steam volatile ..	Present	Present	Present	Present
Solid ..	Present	Nil	Present	Present
Liquid ..	Small amount	Nil	Small amount	Small amount
Resinols—				
$C_{30}H_{50}O$ ..	α-Calotropeol	α-Calotropeol	..	α-Calotropeol
	β-Calotropeol	β-Calotropeol	..	β-Calotropeol
	β-Amyrin	β-Amyrin	β-Amyirin	β-Amyrin
$C_{30}H_{50}O_2$ ..	Giganteol	Nil	Giganteol and isogiganteol	Nil
Sterols ..	Present	Nil	Present	Present
Cardiac poison ..	Very little	Gigantin present	Small quantities	Very little

up of hydrocarbons, the true esters being negligible in amount. Latex and root bark are conspicuous for the absence of these components. The aliphatic waxy portion obtained from the flowers differs from that of the stem bark in consisting mostly of esters to an almost complete exclusion of free acids and hydrocarbons.

On the other hand aliphatic acids occur throughout the plant in combination with resinols as esters. In this respect there is resemblance among the different parts except the latex. Even in the case of the latex, representatives of this type could be found in the volatile acids but the complete absence of both solid and non-volatile liquid fatty acids is noteworthy. The stem bark seems to be the most prolific source for the production of aliphatic components both in variety and in yield. It may be stated here that a similar difference in the composition of the whole plant and of the latex was noted in the case of *Sonchus arvensis* by Stern and Zellner.[3]

Resinols occur all through the plant in the form of esters. In the latex they occur as esters of steam volatile acids only, whereas in the other parts esters of both steam volatile and non-volatile higher fatty acids are present. Four resinols are found in the stem bark. Only three of them could be isolated from the latex and flowers. Though the root bark also yields three resinols, it differs sharply from the rest in containing predominantly giganteol and isogiganteol, the latter of which is found to be present only in this part of the plant. The difference is further accentuated by the complete absence in the roots of calotropeols which are evenly distributed throughout the rest of the plant.

Sterols seem to be absent in the latex whereas their presence has been invariably noted in the other parts of the plant. The latex is the best source for the isolation of the cardiac poison of the usharin type. It is present only in minute quantities in the stem bark and in slightly better proportion in the root bark.

SUMMARY

The ligroin extract of the flowers yielded waxy matter. One fraction of it consisted mainly of the esters of the resinols, α- and β-calotropeols and β-amyrin, with volatile as well as long chain fatty acids. There was also some sterol. The non-resinol aliphatic part contained mainly esters of wax acids and alcohols. The alcoholic extract of the wax-free flowers yielded only mineral matter—citrates, chlorides and tartrates of sodium and potassium.

The various parts of *Calotropis gigantea* are compared with regard to the chemical components present in them.

REFERENCES

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