

# CHEMICAL COMPOSITION OF *CALOTROPIS GIGANTEA*

## Part II. Wax and Resin Components of the Stem Bark

By P. BHASKARA RAMA MURTI AND T. R. SESHADRI

(From the Departments of Chemistry and Chemical Technology, Andhra University)

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IN Part I the examination of the milky latex of the plant was described.<sup>1</sup> The stems have not been examined in the past. They are to some extent used for extracting the useful fibre. Preliminary experiments indicated that the woody portions contribute very little to the total quantity of wax and resin and that they are concentrated in the fibrous bark. Extraction with ligroin and subsequently with ether removed all the waxy components which amounted to about 4% of the dry bark. The subsequent alcoholic extract consisted only of mineral matter.

By digesting with a large volume of alcohol the waxy matter was separated into two major fractions, (A) sparingly soluble solid and (B) alcoholic solution. By direct crystallisation of fraction (A) no crystalline entity could be isolated. It was therefore saponified and the unsaponifiable matter subjected to fractionation. Two main fractions were studied in this case also, a crystalline solid ( $A_1$ ) and the mother liquor ( $A_2$ ). Direct crystallisation of ( $A_1$ ) was again unsuccessful in yielding a sharp melting definite compound. It gave colour reactions characteristic of calotropeols. It was therefore acetylated and the product repeatedly recrystallised from ethyl acetate. The top fraction consisted of a definite compound melting at 250–52° and having the specific rotation of +98° in benzene solution. It had the formula  $C_{32}H_{52}O_2$ . On hydrolysis it yielded a triterpene alcohol of the composition  $C_{30}H_{50}O$  and the specific rotation +102.0° in benzene solution. The properties of these corresponded closely with those of  $\alpha$ -calotropeol acetate<sup>1</sup> and  $\alpha$ -calotropeol respectively; the crystal structures agreed and the mixed melting points were undepressed. The identity was confirmed by preparing the benzoate which melted at 273–74° and was found to be identical with  $\alpha$ -calotropeol benzoate.

When ( $A_1$ ) was benzoylated and the mixed benzoates repeatedly recrystallised a substance melting at 279–80° was obtained. The free alcohol derived from it melted at 216–17°. The structure of the crystals and

the specific rotations agreed with those of  $\beta$ -calotropeol benzoate and  $\beta$ -calotropeol respectively and the identity was established by careful comparison and mixed melting point determination. The melting point of the acetate prepared from this alcohol and its specific rotation agreed with those of  $\beta$ -calotropeol acetate.

When the mother liquor ( $A_2$ ) was evaporated it yielded an amorphous solid which too could not be purified by simple crystallisation. However, after acetylation and repeated recrystallisation of the product, a pure substance melting sharp at  $239^\circ$  could be isolated. It gave colour reactions for pentacyclic triterpenes and had the formula  $C_{32}H_{52}O_2$ . On hydrolysis it yielded an alcohol melting at  $196-97^\circ$ . The acetate and the alcohol were found to be identical with  $\beta$ -amyrin acetate and  $\beta$ -amyrin respectively by comparison with authentic samples obtained from the roots of *Decalepis Hamiltcnii*.<sup>2</sup> By carefully working up the mother liquors left from  $\beta$ -amyrin acetate another fraction melting at about  $140^\circ$  could be obtained. Its composition also indicated that it consisted of triterpenes. The colour reactions, however, were different from those of the definite compounds described above; with both the Liebermann-Burchard and Salkowski reagents it produced yellow solutions with green fluorescence. This could be taken as indication that this fraction contained tetracyclic triterpenes. The surmise seemed to be supported by the observation that when its chloroform solution was treated with hydrogen chloride, a new product melting at  $210^\circ$  was obtained and this gave the correct colour reactions for pentacyclic triterpenes. The melting points of the tetracyclic fraction and of its transformation product were somewhat indefinite. It is obvious, therefore, that they were mixtures. Their separation was not possible since the available material was very small in amount. The most soluble fraction of the acetates that could be obtained from the last mother liquors gave tests for the presence of sterols and it melted between  $130$  and  $135^\circ$ . The quantity, however, was very small.

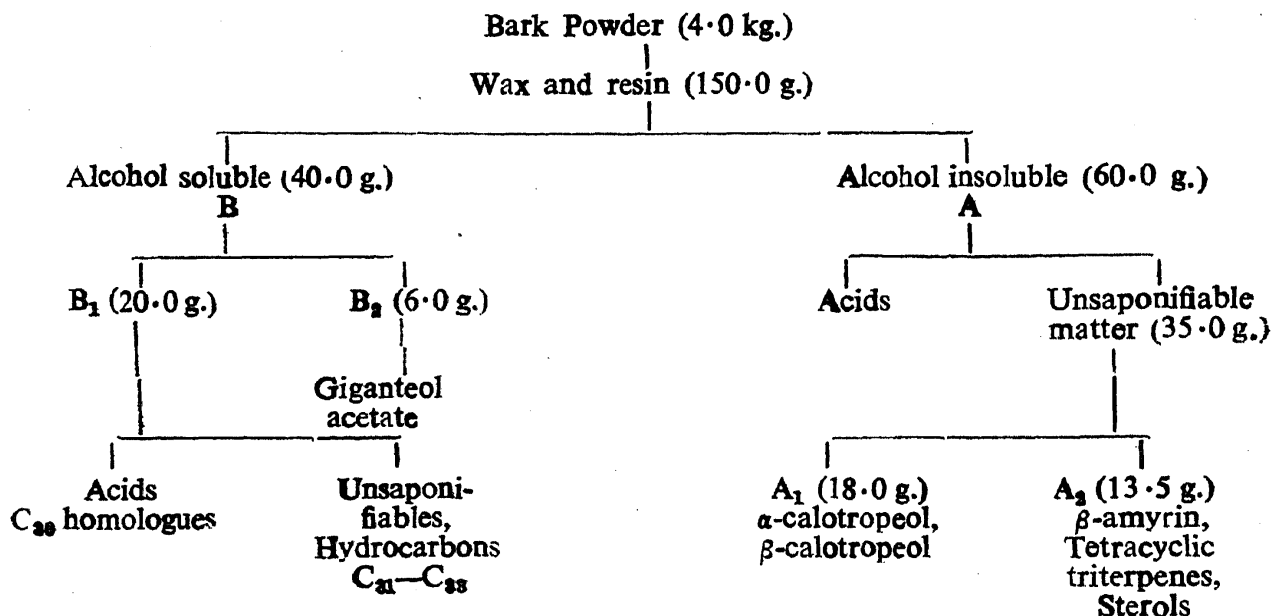
The unsaponifiable portion of fraction (A) seemed to be composed mainly of  $\alpha$ - and  $\beta$ -calotropeols, lesser amounts of  $\beta$ -amyrin, very small quantities of tetracyclic compounds and probably traces of sterols. By the decomposition of the soap from fraction (A) was obtained a mixture of fatty acids. It consisted of 90% solid and 10% liquid acids; the solid portion contained mostly  $C_{30}$ - $C_{32}$  acids and near homologues along with a small proportion with shorter chain lengths. Fraction (A) therefore consisted of esters of the above-mentioned resinols with these acids.

The alcoholic solution (B) was concentrated and the waxy matter collected in two fractions, ( $B_1$ ) the less soluble fraction and ( $B_2$ ) the more

soluble fraction. The former did not give any colour with Liebermann-Burchard or Salkowski reagents. The saponification and the neutralisation values and the study of the unsaponifiable matter indicated that it was a mixture of long chain ( $C_{30}$  mostly) fatty acids (90%) and ( $C_{31} + C_{33}$ ) hydrocarbons<sup>3</sup> (10%).

Fraction ( $B_2$ ), the part which was most highly soluble in alcohol, consisted mainly of triterpene compounds. It was repeatedly recrystallised from ether and ethyl acetate and an ivory white crystalline substance melting at  $252^\circ$  was finally isolated. It had the composition corresponding to the formula  $C_{32}H_{52}O_3$ ;  $[\alpha]_D^{28}, +103.8^\circ$  in benzene. That it was an acetate could be surmised from the formula and it was established by its hydrolysis whereby the corresponding alcohol melting at  $224^\circ$  and having the formula  $C_{30}H_{50}O_2$  could be obtained. The alcohol had the specific rotation of  $+110.9^\circ$  in benzene. The new acetate was quite distinct from the acetates of  $\alpha$ - and  $\beta$ -calotropeols and of  $\beta$ -amyrin and the nature of the alcohol obtained was also different from that of calotropeols and  $\beta$ -amyrin. Hence the free alcohol was given the name of giganteol and its acetate which occurs free in the waxy matter becomes giganteol acetate. This seems to be definitely different from other known compounds of the triterpene series. The analytical results along with a determination of the acetyl value indicated that the acetate was a mono-acetate and the alcohol a dihydric alcohol.

The various stages in the study of the wax and resin components of the stem-bark are summed up in the following chart.



#### EXPERIMENTAL

The plants were collected from the waste lands of Waltair and when fresh the bark was peeled out, dried and powdered. A small sample was

extracted using different solvents in succession; particulars about the extracts are given below.

Solvent	Yield	Nature of the extract
Ligroin ..	4.2%	Waxy matter containing resinols
Ether ..	0.4%	Waxy matter
Chloroform ..	0.2%	Do.
Alcohol ..	3.2%	Inorganic salts

For purposes of detailed study ligroin and ether extracts were mixed together and extraction with chloroform was omitted.

#### *Ligroin + Ether extract*

4.0 Kilograms of the bark powder were repeatedly extracted in a continuous extractor with ligroin (b.p. 80–110°) in batches of 1 kg. for 20 hours each. Afterwards the powder was percolated with ether and the resulting extract was added on to the ligroin extract. When the solvents were completely removed about 150.0 g. of residue was obtained. It was boiled under reflux with one litre of 96% alcohol for 3 hours. While hot a dark viscous mass separated out at the bottom and it solidified when the contents were cooled to about 60°. At that temperature the supernatant liquid was carefully decanted off and the insoluble residue was again boiled with small portions of alcohol. All the alcohol solutions thus obtained were united (2000 c.c.). The alcohol insoluble portion (60.0 g.) was marked (A) and the alcoholic solution (B).

#### *Alcohol insoluble portion (A). (Esters of resinols with higher fatty acids):*

It was a highly coloured sticky solid melting at 55–60° and a definite substance could not be obtained by simple crystallisation. So it was saponified and the resulting products were studied in detail. The solid (60 g.) was dissolved in benzene (700 c.c.); to the solution was added 8% alcoholic potash (1000 c.c.) and the mixture was boiled under reflux for 15 hours. Then the solvents were distilled off as far as possible and the contents were transferred to an evaporating basin, impregnated with pumice stone and rapidly dried to a friable mass by heating on a water-bath. The dry material was packed in a Soxhlet extractor and extracted with petroleum ether for about 8 hours. By this time all the unsaponifiable matter was extracted. On removing the solvent completely the residue (35.0 g.) was obtained as a colourless crystalline solid mixed with an yellow amorphous product. For purposes of fractionation it was dissolved in boiling alcohol (700 c.c.) and the solution was set aside undisturbed overnight. A clean

white solid was deposited at the bottom of the flask. It was filtered carefully under gentle suction and was washed with small quantities of alcohol to remove the adhering yellow matter. The solid thus obtained was designated (A-1) and the solution (A-2).

*Solid (A-1) ( $\alpha$ - and  $\beta$ -Calotropeols)*

It was a colourless crystalline (needles) solid melting indefinitely between 150° and 175°. With Liebermann-Burchard reagent it gave pink solution which changed to purple on standing. With the Salkowski reagent it produced an yellow solution exhibiting green fluorescence. The material was studied both by acetate and benzoate methods.

*Acetate method. ( $\alpha$ -Calotropeol acetate)*

The solid (10.0 g.) was dissolved in acetic anhydride (40.0 c.c.) and sodium acetate (15.0 g.) was added to it. The contents were boiled under reflux on an oil-bath for 4 hours and then thrown into a large quantity of water and cooled in the refrigerator overnight. The separated solid was recovered by filtration under suction and was washed free of acid with water. It was dried in a steam oven, dissolved in boiling ethyl acetate (300 c.c.) and filtered. On cooling the filtrate a good amount of a crystalline solid readily separated out. After keeping the contents undisturbed for 4 hours, the solid was recovered by filtration. It appeared as plates and needles under the microscope and melted at 214–30°. It was found to be sparingly soluble in ether. So to effect further separation the solid (5.0 g.) was digested with ether (150 c.c.) and the insoluble solid (1.0 g.) melting at 235–45° was separated by filtration. The ether solution when concentrated by spontaneous evaporation deposited some more crops of crystals melting at 239–50°. All these were mixed together and added to the above fraction melting at 235–45° for purposes of further purification. The final ethereal mother liquor yielded low melting products (215–25°) and these were added to similar fractions obtained in later stages. The mixture of high melting solids (m.p. 235–50°) (2.5 g.) was dissolved in boiling ethyl acetate (150 c.c.) and on allowing the solution to cool slowly, shining crystals (hexagonal plates) gradually began to separate out. They melted at about 248° and one more crystallisation from the above solvent raised the melting point to 250–51°. The filtrate was concentrated to 50 c.c. when a second crop melting at 241–45 was obtained. The original ethyl acetate mother liquor on concentration to about 75 c.c. deposited a solid melting at 222–35°. It was crystallised from ether and the melting point was found to have gone up to 240°. All these solids melting at about 240° were mixed together and repeatedly crystallised from ethyl acetate, when some more of the substance melting

sharp at 250–51° was obtained. It was found to be unaffected by further crystallisation.

All the ethyl acetate mother liquors from the above crystallisation were combined and concentrated in stages. This resulted only in the isolation of inseparable mixtures melting at about 230°. The final filtrates on evaporation to dryness left behind a small quantity of an yellow solid giving the sterol-colour reactions; Liebermann-Burchard reagent gave a pink colour rapidly changing to blue and green and the Salkowski reagent a red colour.

The crystalline substance melting at 251–52° appeared as elongated hexagonal prisms and was found to be identical with  $\alpha$ -calotropeol acetate in its properties, reactions and composition. The mixed melting point was unchanged. This was confirmed by hydrolysing it with hot alcoholic potash. The free alcohol was found to agree with  $\alpha$ -calotropeol in all respects and the mixed melting point was undepressed. The correct melting point of this alcohol is 220–21° and not 204–05° as previously recorded. It is best crystallised from benzene-alcohol mixture which purifies it more completely. The benzoate of this sample was also made and was found to agree with  $\alpha$ -calotropeol benzoate in all its properties. The mixed melting point was undepressed.

#### *Benzoate Method ( $\beta$ -Calotropeol benzoate)*

The crystalline solid (A-1) (8.0 g.) was dissolved in dry benzene (60 c.c.), benzoyl chloride (20 c.c.) and dry pyridine (15 c.c.) were added and the mixture was set aside for 12 hours. After heating under reflux on a water-bath for 3 hours, the solvents were distilled off as far as possible under low pressure. The residue was treated with water (300 c.c.) and shaken well with ether (500 c.c.). It was observed that some solid remained undissolved both in the ether and aqueous layers. So benzene (100 c.c.) was added to the mixture and shaken well when the solid went into the benzene-ether layer. It was washed with 1% aqueous sodium hydroxide, dilute sulphuric acid and water in succession. The benzene-ether solution was dried over anhydrous sodium sulphate and concentrated to about 150 c.c. by distillation. On allowing the solution to stand for some hours, a colourless solid melting at about 250° was obtained. The mother liquor was evaporated to dryness and the residue recrystallised from ether (750 c.c.). The resulting solid melted at about 250° and was added to the above one (5.0 g.). The ether filtrate was studied separately. The above benzoate was digested with ether (250 c.c.) and the undissolved portion melting at 274–76° was separated. The ether solution was concentrated in stages when some more of the above solid was obtained (total 2.5 g.). This was dissolved

in ligroin and cooled in the ice-chest for 4 hours when long, glistening crystals melting at  $276-80^{\circ}$  were obtained. On repeated recrystallisation from the same solvent (6 times) a pure compound melting at  $279-80^{\circ}$  was obtained and it was found to be the benzoate of  $\beta$ -calotropeol. On working up the ligroin mother liquors some more of the above solid was obtained.

The original ether filtrate was evaporated to dryness and the residue digested with methyl alcohol. The resulting solid melted at about  $185^{\circ}$ . This was repeatedly crystallised from ligroin and other solvents. The resulting product melted round about  $210^{\circ}$ , and was found to be an inseparable mixture of benzoates.

The identity of the above-mentioned product as the benzoate of  $\beta$ -calotropeol was established not only by comparing it with an authentic sample obtained from the latex but also by saponifying it and preparing the free alcohol and the acetate from it and comparing these also with authentic samples.

*Alcoholic Solution (A-2) ( $\beta$ -amyrin, tetracyclic resinols and sterols)*

The solution containing the more soluble portion of the unsaponifiable matter on concentration did not deposit any crystalline solid. The solvent was completely removed by distillation and the residue was obtained as a yellow sticky mass (13.5 g.). It was dissolved in acetic anhydride (50 c.c.) and sodium acetate (10 g.) was added and the contents were boiled on an oil-bath for 4 hours. They were then largely diluted with water, allowed to stand overnight and then extracted with ether (400 c.c.).

*$\beta$ -Amyrin acetate*

The ether solution was washed free of acid, dried over anhydrous sodium sulphate and then was concentrated to about 75 c.c. A colourless crystalline solid slowly separated out from the solution. It melted at  $226-30^{\circ}$  and gave the usual triterpene colour reactions. On repeated crystallisation from boiling ethyl acetate a compound was obtained in the form of colourless rods and it melted at  $239^{\circ}$  (yield 2.5 g.). (Found: C, 82.2; H, 11.5;  $C_{32}H_{52}O_2$  requires C, 82.1; H, 11.1%.) It was hydrolysed by boiling its benzene solution with alcoholic potash. The resulting compound was crystallised from alcohol-ether mixture from which it was obtained in the form of needles, m.p.  $196-97^{\circ}$ . It was identified as  $\beta$ -amyrin by taking a mixed melting point with an authentic sample obtained from *Decalepis Hamiltonii*.<sup>2</sup> The melting point of the acetate was also undepressed when mixed with  $\beta$ -amyrin acetate. Hence the presence of  $\beta$ -amyrin in this fraction was established. The ethyl acetate mother liquors from  $\beta$ -amyrin

acetate on further concentration yielded impure mixtures melting at about 218°.

The original ether filtrate from which the crude  $\beta$ -amyirin acetate was removed was concentrated in stages. A study of the fractions gave indication for the presence of small amounts of tetracyclic triterpenes and traces of sterols; this has been mentioned in the introductory part of this paper.

#### *Fatty acids*

The soap which was obtained as the result of saponifying fraction (A) was decomposed by heating with dilute sulphuric acid. The liberated fatty acid mixture was ether extracted and the ether distilled off from the extract. The residue (23.0 g.) was brown in colour and melted at about 65–70°. When separated by means of the lead salt-ether method, it was found to consist of about 90% of solid and 10% of liquid fatty acids. The solid acid mixture on crystallisation from alcohol several times yielded a fraction (15.0 g.) melting at 83–85° and having a saponification equivalent of 472.8. Consequently it was considered to consist mostly of acids having  $C_{30}$ – $C_{32}$  chain lengths. The remaining portion of the solid acids melted indefinitely at 60–65° and had a saponification equivalent, 302.6. This fraction obviously consisted of fatty acids of lower chain lengths.

Fraction (A) should, therefore, consist of esters of resinols with mostly solid fatty acids of longer as well as shorter chain lengths and with smaller amounts of liquid fatty acids also.

#### *Alcoholic solution (B)*

On keeping this undisturbed overnight a white nodular solid separated out and it was collected by filtration at 70° using a hot water funnel (40 g.). It produced a slightly yellow solution with sulphuric acid. This was again dissolved in alcohol (750 c.c.) and allowed to crystallise. The crystals now melted at 85–120°. After one more crystallisation from ethyl acetate (500 c.c.) a colourless wax producing no colour with sulphuric acid, was obtained (20 g.). This was designated solid ( $B_1$ ). The original alcoholic filtrate and the subsequent ethyl acetate filtrate on concentration yielded high melting solids, m.p. 170–200°. This fraction was designated ( $B_2$ ) (6.0 g.). The filtrate from solid ( $B_2$ ) on evaporation to dryness deposited a small quantity of fatty oil.

#### *Solid ( $B_1$ ) (Fatty acids and hydrocarbons)*

This was sparingly soluble in cold alcohol and in ether but dissolved in large volumes of hot alcohol, chloroform, benzene and petroleum ether. The clean solid (10 g.) was crystallised from a mixture of benzene and

alcohol. The resulting product melted at about  $87^{\circ}$ , was insoluble in acids and alkali and did not produce any colour reactions with sterol colour reagents.

Saponification equivalent	..	..	473.1
Neutralisation equivalent	..	..	472.2

### *Saponification*

The solid (10 g.) was dissolved in benzene (300 c.c.), an equal amount of 8% alcoholic potash was added and the mixture was boiled under reflux for 15 hours. The major bulk of the solvents was removed by distillation, the concentrate was mixed with pumice stone and quickly dried to a friable mass. This was extracted in a Soxhlet with petroleum ether and dry acetone in succession. The petroleum ether extracted all the unsaponifiable matter (1.0 g.) leaving nothing for acetone to extract.

### *Unsaponifiable matter (Hydrocarbons)*

This formed 10% of the wax ( $B_1$ ) and about 5.0 g. of the substance were prepared for study. It was yellow in colour and melted at about  $65^{\circ}$ . By repeated crystallisation from petroleum ether and alcohol, a small quantity of a hydrocarbon mixture melting at  $69^{\circ}$  could be obtained. The yield by this method of purification was very poor. Hence all the fractions were mixed together and treated as follows. The solid was dissolved in hot amyl alcohol (30 c.c.), to the solution was added concentrated hydrochloric acid (25 c.c.) and the contents were heated to boiling. On gradual cooling a solid cake was formed on the top of the liquid surface. When subjected to treatment with acetic anhydride and sodium acetate it was recovered unaffected. It was then crystallised twice from alcohol and petroleum ether in succession. The resulting substance (90% yield) appeared as rhombs, melted at  $69^{\circ}$  and set to a solid at  $68.5^{\circ}$ . No change was produced by treatment with sulphuric acid at  $130^{\circ}$  (Found: C, 85.2; H, 14.8%). From the melting point of the mixture the average chain length was found to be 31.5 carbon atoms using the curves of Piper *et al.*,<sup>3</sup> correlating the melting points and the chain lengths of hydrocarbons. It was considered to be most probably a mixture of  $C_{31}$  and  $C_{33}$  hydrocarbons.

### *Fatty acids*

On decomposing the soap with sulphuric acid, the fatty acids were obtained in the form of a cake. The dry solid was dissolved in a mixture of boiling alcohol and benzene (1:1), the contents were concentrated and allowed to stand for 12 hours. A colourless crystalline solid (8.5 g.) melting at  $88.5-89^{\circ}$  slowly separated out. It was sparingly soluble in ether and

alcohol in the cold, but dissolved in benzene, chloroform and ligroin. It had the mean molecular weight of 458.7. (Found: C, 79.4; H, 12.9;  $C_{30}H_{60}O_2$  requires C, 79.7; H, 13.3%). The mixture therefore consisted of  $C_{30}$  acid and its near homologues.

#### *Solid (B<sub>2</sub>) (Giganteol acetate)*

Fraction (B<sub>2</sub>) produced with the Liebermann-Burchard reagent a pink solution and was therefore different from the aliphatic components present in B<sub>1</sub>. For further purification it was dissolved in boiling ethyl acetate (200 c.c.) and the solution was allowed to stand. A lustrous crystalline solid (3.0 g.) melting at 212–22° separated out. On further crystallisation from ethyl acetate it melted at 230–40°. This solid (0.5 g.) was digested with boiling ether (100 c.c.), allowed to stand for some time and the insoluble portion was separated by filtration. On repeated crystallisation from ethyl acetate it had the appearance of flakes and melted at 252°. A carefully crystallised sample had the appearance of hexagonal plates. The mixed melting points with  $\alpha$ - and  $\beta$ -calotropeol acetates were considerably depressed. (Found: C, 79.5; H, 11.3;  $C_{32}H_{52}O_3$  requires C, 79.3; H, 10.8%.)  $[\alpha]_D^{25} + 103.8^\circ$  in benzene solution. (Found: Iodine value, 56.4;  $C_{32}H_{52}O_3$  requires for one double bond, 52.5.)

#### *Giganteol*

The above compound was hydrolysed by boiling with alcoholic potash. The mixture was diluted with water, the alcohol was distilled off as far as possible and the resulting solid was then filtered and crystallised from alcohol. It was obtained in the form of rods and narrow rectangular plates melting at 224°. Mixed melting points with  $\alpha$  and  $\beta$ -calotropeols were considerably depressed and hence it was considered to be different from them. However, it produced colour reactions similar to those of calotropeols. (Found: C, 80.9; H, 11.6;  $C_{30}H_{50}O_2$  requires C, 81.4 and H, 11.3%.)  $[\alpha]_D^{25} + 110.9^\circ$  in benzene solution. (Found: Iodine value, 54.1;  $C_{30}H_{50}O_2$  requires for one double bond, 57.5.)

The potassium soap on decomposition with acids gave tests for acetic acid. Hence the original compound melting at 252° should be considered to be giganteol acetate.

#### *Alcohol Extract (Mineral Matter)*

The stem bark (2.0 kg.) after extraction with ligroin and with ether was repeatedly extracted in the Soxhlet with 3 litres of alcohol for 20 hours. On concentrating the solution to about 500 c.c. and setting it aside for a day a white crystalline solid collected at the bottom of the flask. When filtered

and washed with warm alcohol, it became colourless and formed 2.5% of the powder taken. It was further purified by dissolving in water and removing the small amount of insoluble impurities. The filtrate was then treated with twice its volume of alcohol and cooled in ice. A colourless crystalline solid soon collected at the bottom of the flask and was recovered by filtration. It was found to be made up mostly of metallic salts and consisted of citrates and chlorides of potassium and sodium. Traces of tartrates were also detected. From the alcoholic and aqueous filtrates no crystalline organic substances could be isolated. The presence of a little tannin could be inferred from the ferric chloride reaction.

#### SUMMARY

A study of the wax and resin components of the stem bark of *Calotropis gigantea* is described. They were separated into fractions by means of alcohol. The most sparingly soluble fraction consisted of esters of resinols with long chain (saturated and unsaturated) fatty acids. From the unsaponifiable matter by fractionating the acetates and the benzoates,  $\alpha$ - and  $\beta$ -calotropeols and  $\beta$ -amyrin were obtained. Small quantities of a mixture of possibly tetracyclic triterpene compounds and traces of sterols were also found to be present. A subsequent fraction was aliphatic being made up of 90% of fatty acids ( $C_{30}$  and near homologues) and 10% of hydrocarbons ( $C_{31} + C_{33}$ , etc). The most soluble fraction contained esters of triterpene alcohols with steam volatile fatty acids. By a process of direct crystallisation applied repeatedly a new substance, Giganteol acetate, could be obtained from this fraction. It yielded a new dihydric alcohol, Giganteol, having the formula  $C_{30}H_{50}O_2$ .

An alcoholic extract of the wax-free stem bark yielded mineral matter consisting of citrates, chlorides and tartrates of sodium and potassium.

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