WAX AND RESIN COMPONENTS OF *CALOTROPIS GIGANTEA*

Part III. Root Bark

By P. Bhaskara Rama Murti and T. R. Seshadri

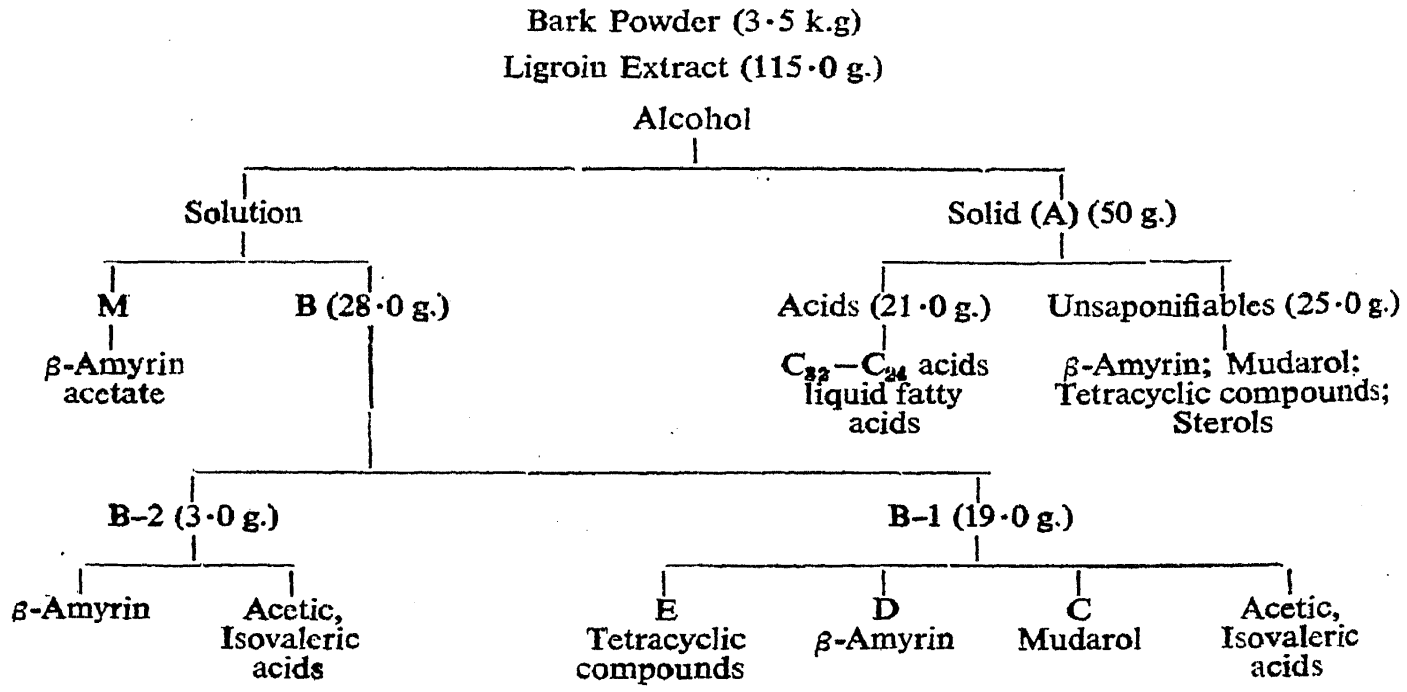
(From the Departments of Chemistry and Chemical Technology, Andhra University)

Received March 6, 1945

In previous publications[1] the chemical components of the latex and stem bark were described. The root bark is also used to some extent as a plant drug and as a poison. This material was previously examined by Hill and Sirkar.[2] The extract obtained by boiling with 95% alcohol was separated by them into four fractions: (1) an oily substance, (2) a white solid, (3) a substance looking like gutta-percha and (4) a yellow bitter principle. From (1), (3) and (4) no chemical entity could be obtained. Though the bark yielded a gutta-percha like substance, the authors did not consider it to be chemically related to gutta-percha. They did not find any caoutchouc. On the other hand, fraction (2), the white solid, when repeatedly crystallised from alcohol, yielded two products, (A) white nodular crystals melting at 140° and (B) white needle-like crystals melting at 210°. (A) was given the formula $C_{35}H_{56}O_3$ and was found to be an ester. By the saponification of (A) with potash a crystalline alcohol melting at 176° was obtained. This was named mudarol and was found to have the formula $C_{30}H_{48}O_2$. Its acetate had the melting point 195–96°. By the oxidation of this alcohol with chromic acid mudaric acid ($C_{30}H_{46}O_3$) was obtained. As the other product of saponification isovaleric acid was isolated and consequently (A) was considered to be the isovalerate of mudarol. Analysis of the needle-shaped crystals melting at 210° (B) agreed with the formula $C_{43}H_{70}O_3$. On saponification it yielded besides potassium isovalerate, a new alcohol melting at 215°. It was given the formula $C_{38}H_{62}O_2$ and the name akundarol. Its acetyl derivative melted at 222°. The substance (B) was therefore considered to be akundarol isovalerate. Akundarol also was reported to give an acid, akundaric acid on oxidation with chromic acid. These authors recorded that both these alcohols and their isovalerates gave colour reactions similar to those of sterols and they considered that they were quite different from resin alcohols isolated by Cohen and Van Romburgh from various latices and resins. (The names of the compounds are derived from the Hindi and Bengali names of the plant.)

The dried root bark has now been examined on the same lines as the stem bark[1] already described. The ligroin extract contained almost the entire wax and resin components. It was separated into three main fractions using alcohol: fraction (A), least soluble in alcohol; fraction (B), middle fraction; and fraction (M) (mother liquor). Since no definite substance could be obtained from (A) and (B) by repeated crystallisation, they were separately saponified.

The unsaponifiable matter from (A) had all the characteristic properties of triterpene alcohols. By repeated fractional crystallisation a crystalline solid melting sharp at 176–7° and having a characteristic appearance (hexagonal plates) could be isolated. It was a dextrorotatory alcohol, its composition agreeing with the formula $C_{30}H_{48}O_2$. Its colour reactions were similar to those of the calotropeols, and different from those given ordinarily by sterols. Further it did not form a digitonide. Since its crystal structure, melting point and composition agree with those given for mudarol and since it seems to be a definite entity we propose to retain the original name mudarol for this substance though there seem to be differences in the description of colour reactions and derivatives and it seems to be definitely a resinol. Besides this substance, β-amyrin could also be obtained. In the tail fractions there were small quantities of substances which could be considered to be tetracyclic triterpenes and sterols as obtained in similar fractions in the stem bark; but the quantities were very small. The acid portion consisted mostly of long chain solid fatty acids (C_{24}–C_{32}) and small amounts of liquid fatty acids. Thus (A) consisted mostly of esters of the triterpene alcohols, mudarol and β-amyrin with these non-volatile fatty acids.



Fraction (B) was found to consist almost completely of esters of the above alcohols with volatile fatty acids. Mudarol and β-amyrin could again be isolated in definite quantities from this fraction also. From (M) free β-amyrin acetate could be isolated by simple crystallisation thus confirming that acetates were present in the esters. The details of the study of the wax and resin components are diagrammatically represented above.

The subsequent ether and chloroform extracts of the root bark seemed to contain small amounts of cardiac poisons giving tests for sulphur. The alcoholic extract gave indications for the presence of tannins. Otherwise no definite components could be obtained from them.

In the course of our investigation of the root bark no substance corresponding to the description of akundarol of Hill and Sirkar could be obtained though we made several experiments and looked for it. The reported properties of this substance do not agree with β-amyrin which is definitely found in the roots and which they missed . The gutta-percha like substance of Hill and Sirkar should have consisted of the esters of resinols mixed with tannins and other materials since they used alcohol for extraction. The isolation of β-amyrin in our work shows conclusively that the roots also contain triterpene alcohols.

It may be useful to note here the resemblance between the roots of *Calotropis gigantea* and those of *Decalepis Hamiltonii* and *Hemidesmus indicus* all of which belong to the family Asclepiadacea. The waxy matter in all the three consist mostly of resinol esters of non-volatile and volatile fatty acids and contain negligible amounts of free aliphatic acids, alcohols or hydrocarbons. The following table brings out details:

	D. Hamiltonii	*H. indicus*	*C. gigantea*
1. Pure resinol esters isolated	β-amyrin acetate	β-amyrin acetate	β-amyrin acetate
2. Major components (resinols)	(*a*) β-amyrin (*b*) α-amyrin (*c*) Lupeol	(*a*) β-amyrin (*b*) α-amyrin (*c*) Lupeol	(*a*) β-amyrin (*b*) Mudarol
3. Minor components ..	(*a*) Tetracyclic resinols (*b*) Sterols	Tetracyclic resinols Sterols	Tetracyclic resinols Sterols
4. Acids derived from esters	Acetic ; liquid and solid acids	Acetic ; liquid and solid acids	Acetic ; isovaleric ; liquid and solid acids

Experimental

A large number of the plants of *Calotropis gigantea* were dug out of the earth and the roots were carefully separated from the stems. The root bark

was thick and colourless and could be easily separated from the inner woody portions when fresh. It was dried in the sun and ground into a coarse powder. During drying and storing it was not attacked by fungus or insects.

The coarse powder (3·5 kg.) was subjected to continuous extraction with ligroin (b.p. 80–110°) for about 24 hours and the solution distilled in order to remove the solvent completely. A brownish yellow sticky residue weighing about 115 g. was obtained. It was boiled under reflux twice with rectified spirits employing 500 c.c. of the solvent each time. The clear supernatant solution was decanted off at about 60°. The sticky semi-solid mass left behind was marked (A). The alcoholic solution was concentrated to about 200 c.c. and the solid matter that separated out was marked (B). The mother liquor did not deposit any solid when allowed to stand, for several days; it was marked (M).

Fraction (A): Esters of resinols with higher fatty acids

The solid (50·0 g) melting round about 60° was dark brown in colour and was very sticky. It dissolved in ether, chloroform and benzene easily and was sparingly soluble in methyl and ethyl alcohols. No crystalline component could be isolated from it by fractional crystallisation employing different solvents. Hence it was subjected to saponification using benzene and alcoholic potash according to the procedure described in previous publications and the unsaponifiable matter and the fatty acids were examined.

Unsaponifiable Matter: Resinols

It consisted predominantly of crystalline material mixed up with some yellow amorphous solid (25·0 g). It was dissolved in alcohol (400 c.c.) and the solution slowly concentrated to about (250 c.c.) and allowed to stand over night. The crystalline solid that separated out was filtered off and marked (A-1), similarly fraction (A-2) was obtained after concentration to 150 c.c., (A-3) after concentration to 75 c.c. and (A-4) after concentration to 40 c.c. Fraction (A-5) was the residue left after evaporating the final mother liquor.

Fraction (A-1): (Mudarol)

This fraction (3·0 g.) was in the form of a crystalline powder; under the microscope it appeared as broken plates. After one more crystallisation from alcohol it melted sharp at 176-7° and had a definite crystalline shape (hexagonal plates). No further rise in the melting point and no change in structure could be effected by repeated cystallisation (Found C, 82·1; H, 11·1; $C_{30}H_{48}O_2$ requires C, 81·8; H, 10·9%); $[\alpha]_D^{28°}$, + 92·5° in chloroform solution. (Found: Iodine value, 55·2; $C_{30}H_{48}O_2$ requires for one double bond, 57·5). With the Salkowski reagent it produced a yellow solution exhibiting green flourescence. In the Liebermann-Burchard reaction

addition of a drop of sulphuric acid produced a pink solution which changed to purple in an hour, then slowly to pale blue in about 2 hours and finally faded to yellowish brown. Addition of a few drops of water expedited these changes. It did not react with digitonin.

Fraction (*A*-2): (*Mudarol and β-amyrin*)

The solid (6·5 g.) was found to be a mixture of compounds having two different crystalline structures, hexagonal plates and flat needles. They were picked out as far as possible and separately purified. Mudarol and β-amyrin were found to be the components. The former being markedly less soluble in alcohol came out first during the crystallisations. The hexagonal plates consisted mainly of mudarol and the needles of β-amyrin.

Fraction (*A*-3): (*β-amyrin*)

It crystallised from alcohol in the form of long needles and melted at about 180°. Repeated crystallisation did not yield a pure compound. Hence it (4·0 g.) was subjected to acetylation by boiling with acetic anhydride and sodium acetate for 3 hours. The resulting product was dissolved in ethyl acetate (50 c.c.) and set aside for a day whereby a shining crystalline solid in the form of stout prismatic rods separated out. After one more rapid crystallisation from the same solvent the substance was obtained in the shape of tiny shining rods and melted sharp at 238-39°. When it was mixed with β-amyrin acetate obtained from the latex no depression in the melting point was noticed and hence its identity was established. From the ethyl acetate mother liquors mixtures melting indefinitely at about 220° were obtained and they could not be further purified.

Fraction (*A*-4): (*Resinols*)

This fraction (4·8 g.) appeared as a mixture of colourless crystals and yellow resin. It was subjected to acetylation by boiling with acetic anhydride (15 c.c.) and sodium acetate. The resulting product was crystallised from ethyl acetate (50 c.c.) when a sparingly soluble solid separated out in the form of hard crystals melting at about 230°. The above fraction on further purification by crystallisation from ethyl acetate yielded β-amyrin acetate melting at 238-9°. The original ethyl acetate mother liquor was concentrated in stages and solid products which were found to be impure β-amyrin acetate were removed. The filtrate obtained from above was evaporated to dryness and the residue was crystallised from alcohol. By this operation a small amount of a colourless solid giving a yellow Liebermann-Burchard reaction was obtained. The reaction mixture exhibited an intense green fluorescence and in the course of two hours the colour changed to pink the fluorescence disappearing at the same time. However it melted indefinitely at about 170° and could not be further purified (yield 0·2 g.).

Fraction (A-5)

It was obtained as a sticky, yellow solid (3·0 g.). No crystalline compound could be obtained from it even after acetylation. It gave a blood red colour in the chloroform layer with the Salkowski reagent and a play of colours, pink-blue-green with the Liebermann-Burchard reagent indicating the possible presence of sterols.

Fatty Acids

The acid mixture (21·0 g) (obtained by the decomposition of the soap) was dark brown in colour and melted at about 72°. On treatment by the lead-salt-ether method it yielded 95% solid acids. On crystallising the solid acids from alcohol the top fraction (12·0 g) was obtained in the form of rhombs and it melted at 84-86°. It had a mean molecular weight of 478·2 and therefore seemed to be composed of C_{32} acids and its homologues. The subsequent fraction was found to have a melting point range of 65-69° and a mean molecular weight of 350. Consequently it was considered to be made up of C_{24} acid and its near homologues. The liquid acids recovered were very small in quantity and could not be characterised.

Fraction (B): Esters of resinols with steam volatile fatty acids

This fraction (28·0 g.) was divided into two by digestion with hot alcohol (250 c.c.). The undissolved portion was marked (B-1) and the dissolved portion (B-2).

Fraction (B-1): (Esters of mudarol, β-amyrin and tetracyclic resinols)

This fraction (19·0 g.) was a mixture of some colourless crystalline and of some yellow amorphous substances. It melted at about 160° and produced the usual pink solution with the Liebermann-Burchard reagent and yellow solution with the Salkowski reagent. It was subjected to saponification by boiling its benzene solution (500 c. c.) with 8% alcoholic potash for 15 hours. The major bulk of the solvents was then distilled off and the concentrate was largely diluted with water and reapeatedly ether extracted. All the ether solutions were united and dried.

The soap solution was heated to remove the last traces of the solvents, acidified with sulphuric acid and steam distilled. The aqueous distillate had an offensive smell suggesting the presence of isovaleric acid. The presence of acetic acid was also detected in the distillate by making use of the lanthanum nitrate test. The aqueous sulphuric acid solution on ether extraction did not yield any non-volatile fatty acid.

The unsaponifiable matter was digested with boiling alchol (400 c. c.) and set aside for 12 hours. A good crop of hard crystals (7·5 g.) settled

down and they were separated by filtration. This fraction was marked (C). The alcoholic solution was concentrated to about 100 c.c. and the fraction that separated was marked (D). The remaining mother liquor constituted fraction (E).

When fraction (C) was crystallised twice from alcohol, it yielded a homogeneous crystalline solid having the characteristic crystal appearance of mudarol (hexagonal plates) and melting at 176-77°. It was found to be identical with the sample of mudarol already described.

Fraction (D) (4·5 g.)

It appeared as long needles under the microscope and produced a pink solution with the Liebermann-Burchard reagent. It melted at about 160° and could not be further purified by simple crystallisation from different solvents. Hence it was subjected to acetylation by boiling with acetic anhydride in presence of sodium acetate. The crude acetate which was coloured, was dissolved in benzene (75 c. c.) and decolourised with a pinch of animal charcoal. To the benzene solution an equal volume of alcohol was added and the mixture was concentrated to about 100 c. c. As the result a hard gritty crystalline solid melting at 185-90° separated out and after one more crystallisation from ethyl acetate its melting point rose to 220-25°. After two further crystallisations from the same solvent it yielded a small amount of substance melting at 238°. It was found to be identical with β-amyrin acetate by taking a mixed melting point. The mother liquors obtained from the crude β-amyrin acetate on concentration yielded crops melting at 160-190°. And no pure substance could be obtained from them by crystallisation. Fraction (D) thus seemed to be consisiting predominantly of β-amyrin.

Fraction (E)

It yielded an amorphous residue (3·0 g.) which was studied by the acetate method. On crystallising the acetates from ether, the top fractions contained β-amyrin acetate and the pure substance could be obtained by recrystallisation. The tail fractions yielded a small quantity (0·2 g.) of a colourless substance melting round about 162°. Even after repeated recrystallisation from alcohol the melting point was not definite (Found: C, 81·9; H, 10·9; $C_{32}H_{52}O_2$ requires C, 82·1; H, 11·1%); $[\alpha]_D^{28°}$, + 32·0 in chloroform solution. It produced a yellow solution with intense green fluorescence in both Liebermann-Burchard and Salkowski reactions. When it was treated in chloroform solution with dry hydrogen chloride at 0° the product melted round about 220° and gave colour reactions characteristic of penta-cyclic triterpenes (Liebermann-Burchard reaction, pink). The

possible presence of tetracyclic triterpene alcohols in this fraction was thus indicated.

Fraction (B-2) (3·0 g.)

This was also saponified and the unsaponifiable matter repeatedly crystallised from alcohol. A sharp melting pure substance was thus obtained. It was identified as β-amyrin by comparison with an authentic sample and conversion into its acetate melting at 238°. The fatty acid portion was found to consist of steam volatile fatty acids.

Mother liquor (M): (β-amyrin acetate)

The original alcoholic mother liquor obtained after filtering off solid (B) was distilled to recover most of the solvent. The concentrated solution (50 c.c.) was very viscous. It was set aside for a number of days to facilitate crystallisation. Slowly a granular solid began to separate out and its deposition was hastened by keeping the contents in an ice-chest. It was found to be a mixture of a crystalline component and an amorphous yellow solid; the latter was removed by repeated washing of the mixture with alcohol. The colourless solid thus obtained (4·0 g.) had a melting point range of 195-210°. To effect further purification it was dissolved in boiling ethyl acetate (75 c.c.) and the solution was allowed to cool slowly whereby a crystalline solid melting at about 225° was obtained. After one more crystallisation from the same solvent its melting point was raised to 238-39° and the pure substance had the appearance of narrow prismatic rods. On hydrolysis with alcoholic potash it yielded an alcohol melting at 195°. The acetate and the free alcohol were found to be identical with authentic samples of β-amyrin acetate and β-amyrin.

Summary

The wax and resin components of the root bark of *Calotropis gigantea* have been examined. A substance corresponding to the description of 'mudarol' of previous workers could be isolated. However, its properties indicate that it is a triterpene alcohol and not a sterol. β-amyrin is also found as a major component. These two alcohols are present as esters of volatile and non-volatile fatty acids. β-amyrin acetate could be isolated by direct fractional crystallisation. No substance corresponding to the description of 'akundarol' of Hill and Sirkar could be found.

References

1. P. B. R. Murti and T. R. Seshadri — *Proc. Ind. Acad. Sci.*, A, 1943, **18**, 145.
2. ———— .. *Ibid.*, 1945, **21**, 8.
3. Hill and Sirkar .. *J. C. S.*, 1915, 1437.