CHEMICAL COMPOSITION OF *CALOTROPIS GIGANTEA*

Part I. Wax and Resin Components of the Latex

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Calotropis gigantea is a shrub common in the eastern and southern parts of India, Ceylon and Eastern Asia. The milky juice obtained from it is used for medicinal and insecticidal purposes and frequent cases of criminal poisoning by its means are known. The possibilities of obtaining a guttapercha-like solid from this latex have also been considered in the past. Its chemical composition was first investigated by Basu and Nath[1] who employed the dried latex. From the unsaponifiable portion of the ether-soluble matter they isolated, after repeated purification, a product which was considered by them to be a sterol of the formula $C_{28}H_{44}O$. It was named calosterol. They, however, noted that this substance did not form a precipitate with digitonin and in most of its reactions differed from ordinary sterols. As part of their investigations on African arrow poisons G. Hesse and co-workers[2] examined the mixed latex obtained from *Calotropis procera* and *Calotropis gigantea*. From the alcohol-soluble portion were obtained usharin, calotoxin and calactin which belong to the group of cardiac poisons.

Considerable quantities of the latex can be collected easily and the material is best preserved by adding a little chloroform. Some amount of preliminary investigation had to be done in order to work out the most suitable method of separating the components. The procedure adopted by Basu and Nath of drying the whole material so as to obtain a solid does not seem to be satisfactory, since it leads to undesirable decompositions and the separation of the various components from the resulting product is also difficult. Coagulation by simple heating or by treatment with acids with a view to separate fractions is better, but still not adequate. The most satisfactory procedure is to add enough alcohol to produce a filterable precipitate and a clear filtrate, and it renders the separation of the various components more easy. The soft coagulum (I) and the aqueous alcoholic solution (II) were separately examined.

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The coagulum (I) was repeatedly extracted with boiling alcohol and subsequently with ether. It was finally separated into a sticky solid (I *a*), which was difficultly soluble in ordinary solvents and which is still under investigation and a portion easily soluble in ether (I *b*). The above separation rendered the study of the ether-soluble fraction more easy. This was a crystalline solid melting between 120° and 170°. With a view to understand the nature of the components present, colour reactions were carried out using several reagents. The most characteristics reactions were a deep pink colour with the Liebermann-Burchard reagent and a yellow solution with the Salkowski reagent and these indicated the presence of triterpene compounds. With a view to obtain some information about the exact condition in which these compounds occur and if possible to obtain a pure substance repeated crystallisation was carried out. A small amount of a fraction melting at 248–50° (solid A) could be isolated; it will be described later on. The remaining portion was, therefore, subjected to saponification and the unsaponifiable matter and the fatty acids analysed separately.

The unsaponifiable matter was a pale yellow solid and consisted mostly of resinols. It was divided into two fractions using solubility in alcohol: less soluble (B) and more soluble (C). Since no definite entity could be obtained from them by adopting methods of crystallisation, they were independently subjected to acetylation and benzoylation and these esters were subsequently subjected to fractional crystallisation. Such a procedure has yielded very good results in the hands of Heilbron and co-workers in similar cases. The existence of α- and β-amyrins and of lupeol in the waxy portion of *Decalepis Hamiltonii* and *Hemidesmus indicus* could be established by adopting this technique.[3] By the acetylation of (B) and crystallisation of the mixed acetates using ethyl acetate a definite compound melting at 250–51° could be obtained as the major product. Its sparing solubility was noteworthy; its crystal structure (elongated hexagonal plates) was very characteristic and was very sensitive to the presence of impurities; $[\alpha]_D^{32°}$, + 98·0° in benzene solution. With Liebermann-Burchard reagent it produced a pink solution which deepened slowly to purple. The colour faded very slowly and after several hours it appeared to be pale blue and eventually turned yellowish brown. With Salkowski's reagent it produced a yellow solution exhibiting powerful green fluorescence. These colour reactions seemed to be individual characteristics of the substance and also indicated that it belongs to the triterpene group. This surmise was supported by the results of combustion analysis which corresponded to the formula, $C_{32}H_{52}O_2$ and by a molecular weight determination. The latter value was obtained by employing the

saponification equivalent, since it could not be correctly obtained by the camphor method of Rast. Hydrolysis of the acetate gave rise to an alcohol melting at 204–5° $[\alpha]_D^{32°}$, + 102·0° in benzene solution and its composition corresponded to the formula, $C_{30}H_{50}O$. It formed no combination with digitonin. Since it did not appear to be identical with any known compound it has been given the name, α-calotropeol indicating its isolation from Calotropis. Its benzoate melted at 273-74°, $[\alpha]_D^{32°}$, + 74·3° in bezene solution. Thus the alcohol and its esters are all dextrorotatory. From a determination of the iodine value of the acetate the presence of one double bond in the compound could be established. From the mother liquors left after the separation of α-calotropeol-acetate, small quantities of the acetates of another new alcohol, β-calotropeol (see below) and of β-amyrin could be obtained with great difficulty. However, β-calotropeol was more easily obtained in good quantity as its benzoate from the crystallisation of the mixed benzoates produced by the benzoylation of the original resinol mixture (B).

By the repeated crystallisation of the mixed benzoates a substance melting sharp at 279–80° could be isolated. It was different from α-calotropeol benzoate, the mixed melting point being depressed and its specific rotation, $[\alpha]_D^{32°}$, + 69·0° in benzene solution was also different thus showing that they are different substances. However, from combustion analysis and molecular weight determination it was found to be isomeric. The free alcohol obtained from it melted at 216–17° and had the specific rotation, $[\alpha]_D^{32°}$, + 50·9° in benzene solution and was thus different from α-calotropeol. It was, therefore, named β-calotropeol. It resembled α-calotropeol in its composition and colour reactions. The individuality of β-calotropeol was further supported by the preparation of the acetate which was found to melt at 238°, $[\alpha]_D^{32°}$, + 43·9°, and to be quite different from α-calotropeol acetate, the mixed melting point being depressed. It should be mentioned here that the calotropeols were found to be stable and their acetates did not undergo any change on boiling with formic acid.

The more soluble fraction (C) of the unsaponifiable matter was similarly analysed. The acetate method seemed to be the most satisfactory in this case and by the crystallisation of the crude acetates, a pure substance melting sharp at 239–40° could be isolated. It had the formula, $C_{32}H_{52}O_2$ and gave colour reactions characteristic of pentacyclic triterpenes. On hydrolysis it yielded an alcohol melting at 196–97°. The acetate and the alcohol were found to be identical with β-amyrin acetate and β-amyrin respectively by comparison with authentic samples obtained from the roots of *Decalepis Hamiltonii*. The more soluble portion of the acetates yielded finally a fraction

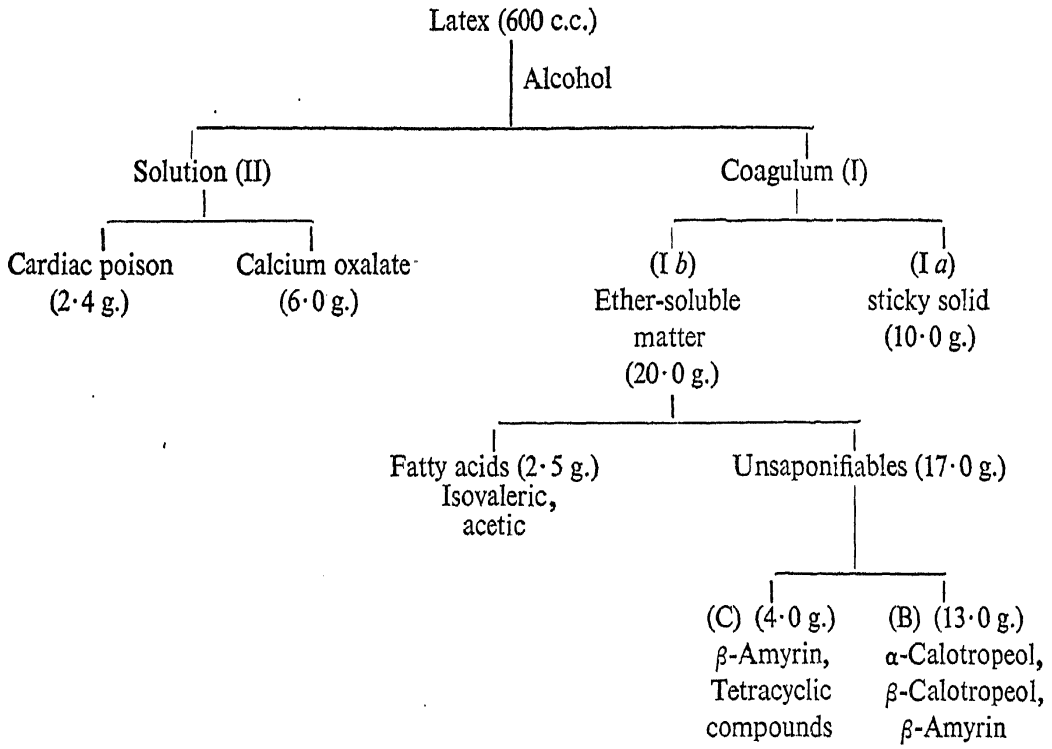
melting at 172–74° and its composition corresponded to the formula, $C_{32}H_{52}O_2$. It was dextro-rotatory. The colour reactions, however, were different from those of the above compounds; with both the Liebermann-Burchard and Salkowski reagents it produced yellow solutions with green fluorescence. This fraction seemed, therefore, to contain tetracyclic triterpenes and this surmise was confirmed by treatment with formic acid or with chloroform saturated with hydrogen chloride. The new product melted between 211 and 215° and gave the colour reactions of the pentacyclic triterpenes (similar to β-amyrin). Thus the presence of mixtures of β-amyrin and tetracyclic resinols was established.

By the decomposition of the soap obtained from the saponification of the ether-soluble portion of coagulum (I) were produced only steam-volatile fatty acids. The existence of acetic acid and of isovaleric acid in the mixture was indicated by the lanthanum nitrate test[6] for the former and the characteristic smell of the latter. An artificial mixture of the two acids could be prepared having practically the same smell as the steam-volatile acids obtained from the latex.

Summing up the results, the ether-soluble portion of the coagulum consists of the esters of triterpene alcohols (acetates and isovalerates). α- and β-calotropeols occur in equal amounts and they form the major portion; β-amyrin comes next and small amounts of tetracyclic compounds also seem to exist.

The aqueous alcoholic solution (II) left after the separation of the coagulum contained substances which could be extracted by means of ether and chloroform. These, after purification using chloroform and petroleum ether, yielded a colourless crystalline substance (mixture of compounds) melting at about 242°. Its colour reactions and solubility indicated that it was not a wax component but belonged to the group of cardiac poisons described by Hesse *et al.* This mixture was highly toxic to fish, gave tests for N and S indicating that compounds containing these elements were present. Further study of this material is in progress. The aqueous alcoholic solution from which all ether- and chloroform-soluble matter had been removed, deposited slowly a colourless solid, and this was identified as calcium oxalate by qualitative and quantitative analyses and examination of its crystalline structure. It is probable that the irritating effect of the latex when rubbed on the skin is due to the presence of this substance in a very fine crystalline condition. This phenomenon has been noticed in some other cases, one such being *Vitis quadrangularis*.[4]

The following chart embodies in brief the isolation of the various fractions and their compositions.



As already mentioned Basu and Nath investigated the ether extract of the dried latex and claimed to have obtained as the sole crystalline component of the unsaponifiable matter a new sterol called calosterol. Though its reactions were abnormal for a sterol they seem to have been led to this opinion by the formula, $C_{28}H_{44}O$ that they obtained for it. However, the carbon analysis of calosterol and of its acetate and benzoate agree closely with the values required for the monohydric triterpene alcohols, $C_{30}H_{50}O$ and their derivatives, though the recorded hydrogen values of the former are low all through. From the results of the present work and particularly from a comparison of the melting points it seems to be quite possible that the above authors were dealing with a mixture of resinols. It was probably contaminated with cardiac poisons since these are also soluble in ether and would get into the ether extract when the whole of the latex in a dry condition is extracted. The cardiac poisons give an immediate green colour with the Liebermann-Burchard reagent and their presence might have contributed to the colour changes observed with calosterol when treated with this reagent.

It may be mentioned in this connection that the latex of another species of Calotropis, *Calotropis procera* has been recently analysed by Hesse[5] *et al.* and has been found to contain a triterpene alcohol. From the abstract of the paper which alone is now available it could be seen that the sole alcoholic component of the latex is α-lactucerol. It has a melting point of 224–25°, $[\alpha]_D$, $+97{\cdot}5°$ and its acetate and benzoate melt at 252° and 257° respectively. On boiling with formic acid it isomerises to iso-lactucerol melting at 201°; $[\alpha]_D^{20°}$, $+66{\cdot}8°$. The acetate and benzoate of iso-lactucerol melt

at 237° and 271° respectively. The alcohols are given the formula $C_{30}H_{50}O$ and they produce colour reactions similar to those noted for the calotropeols. They are described as pentacyclic triterpene alcohols. These observations are in accord with the results of the present investigation of the latex of *Calotropis gigantea*, that the components are resinols though the compounds present in the two species are different. They further support the conclusion that 'calosterol' should be considered to be a mixture of triterpene alcohols.

With regard to the composition of the latex of *Calotropis procera* Hesse *et al.* found it to contain (1) cardiac poisons and (2) esters of α-lactucerol with steam-volatile fatty acids. The present investigation shows that the latex of *Calotropis gigantea* contains (1) cardiac poisons, (2) esters of α- and β-calotropeols and β-amyrin with steam-volatile fatty acids and (3) calcium oxalate. Besides others the most characteristic difference between α-lactucerol and the resinols of *C. gigantea* is that the former undergoes isomeric change when treated with formic acid, whereas the latter are stable to such treatment.

Experimental

Fresh latex was collected in bottles containing a few drops of chloroform. In order to bring about coagulation of colloidal matter, it (600 c.c.) was treated with rectified spirits (200 c.c.), the mixture was well stirred and set aside for some hours. The coagulum (I) (35·0 g.) was separated from the aqueous alcoholic mother-liquor (II) by filtration under gentle suction and the two were examined separately.

The soft coagulum (I) was boiled with alcohol (500 c.c.) under reflux, filtered and the extraction repeated twice again using 250 c.c. of alcohol each time. The residue was then extracted repeatedly with boiling ether till no more went into solution. A small sticky portion (I *a*), pale yellow in colour was left behind; it was difficultly soluble in ordinary solvents, contained a little mineral matter and gave tests for phosphate. The combined alcoholic extract deposited on standing a bulky solid which was completely soluble in ether. It closely resembled the ether extract and was therefore added to it (I *b*). On evaporating the clear alcoholic solution a yellowish brown sticky solid was obtained. It resembled (I *a*) closely and the two were mixed together (10·0 g.). This is under further investigation.

Ether solution, (I *b*) (*Resinol esters,* 20 *g.*).—On distilling off the solvent completely the dry residue was obtained as a colourless, non-sticky solid

melting indefinitely between 120° and 170°. To effect, if possible, the isolation of pure compounds by direct crystallisation, it (25·0 g.) was dissolved in boiling ethyl acetate (300 c.c.) and the solution was left undisturbed for about 6 hours. A glistening mass of colourless crystals melting at 210–15° separated out. Two more crystallisations of this solid (5·0 g.) from the same solvent yielded a product melting at 230–35°. Further repeated crystallisation using ether and ethyl acetate alternately left a small amount of (0·5 g.) of a pure product melting at 248–50° (A). Description of this substance has been postponed for the present.

All the impure fractions were mixed together. The solid (20·0 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. By this time a large amount of a crystalline solid (needles) was found to separate out. The solvents were removed by distillation as far as possible, water was added to the residual mixture and boiled. The unsaponifiable matter was isolated by ether extracting the aqueous suspension; during this operation no emulsions were formed and the soap solution remained clear all the time.

Fatty acids (*acetic and isovaleric acids*).—Before decomposing the soap solution with acids the last traces of alcohol were carefully removed by heating under reduced pressure. This precaution is necessary, since lower fatty acids readily form volatile esters with alcohols in the presence of mineral acids and thus give rise to complications. The acidified solution did not form any layer of insoluble fatty acids and hence the clear solution was subjected to steam distillation. The distillate (2 litres) was ether extracted, the extract dried over anhydrous sodium sulphate and finally distilled to recover the solvent. The liquid residue was strongly acidic in reaction and had an offensive smell. Treatment with lanthanum nitrate[6] produced a blue solution indicating the presence of acetic acid and the smell was characteristic of isovaleric acid. Careful comparison of the odour of a dilute solution of the mixed fatty acids with a similar solution containing authentic samples of acetic and isovaleric acids confirmed the presence of these two acids in the mixture.

The residue in the distilling flask was a clear solution. It was repeatedly extracted with ether and the ether solution evaporated. The absence of any residue showed that solid and liquid non-volatile fatty acids were absent.

Unsaponifiable matter, Resinols (17·0 *g.*).—The ether solution of the unsaponifiable matter was distilled in order to remove the solvent completely. The residue (17·0 g.) was a pale yellow solid containing some colourless crystals. It was readily soluble in benzene, ethyl acetate and

ether, but was much less soluble in both methyl and ethyl alcohols. When the whole solid was boiled under reflux with rectified spirits (400 c.c.) and the contents allowed to stand overnight a crystalline solid (B) separated out. It was filtered off and the alcoholic filtrate concentrated in stages. Since no more crystalline solid could be obtained by this process, the whole of the filtrate was evaporated to dryness and the residue (C) was separately examined.

Solid (B) (13·0 *g.*), *α- and β-calotropeols and β-amyrin.*—This fraction crystallised from alcohol in long colourless needles melting at 120–70°. Simple crystallisation from ordinary solvents a number of times did not yield sharp melting substances. Consequently esters (acetate and benzoate) were prepared and studied.

Acetate method (*α-calotropeol acetate*).—The resinol mixture (8·0 g.) was dissolved in acetic anhydride (20 c.c.), anhydrous sodium acetate (5·0 g.) was added and the mixture was kept boiling under reflux for 3½ hours in an oil-bath. It was cooled, diluted with water (300 c.c.) and kept in the ice-chest overnight. The separated solid was extracted first with excess of boiling ether and subsequently with boiling benzene. Only a little dark resinous matter was left behind and it was discarded. Both the ether and benzene solutions were decolourised by warming with 'norit' and concentrated independently. They yielded the same crystalline solid melting at about 230° which when recrystallised from ethyl acetate melted at 240–44°. The two fractions from the two solvents were mixed together and twice crystallised from ethyl acetate when a compound melting at 250–51° was obtained (0·5 g.).

The crystal structure was quite characteristic; the compound came out slowly in the form of glistening, elongated hexagonal prisms. It was very sparingly soluble in both ethyl and methyl alcohols, moderately soluble in ether and ethyl acetate and readily soluble in chloroform and benzene. With Liebermann-Burchard reagent it produced a pink solution which very slowly faded to a yellow brown. With the Salkowski reagent it formed a yellow solution exhibiting green fluorescence. Treatment with boiling formic acid did not produce any change. [Found: C, 82·5; H, 11·2; $C_{32}H_{52}O_2$ requires C, 82·1; H, 11·1%]. $[\alpha]_D^{30°}$, + 98·0° in benzene solution. The molecular weight of the acetate was determined by finding the saponification equivalent. The solid was dissolved in benzene, excess of N/2 alcoholic potash was added to it and the contents were boiled under reflux for 3 hours. A blank experiment was conducted simultaneously. The unused alkali was estimated by titration with standard acid. The molecular

weight was found to be 462 and that required for the formula given above is 468. The iodine value was determined as follows: The substance (0·2180 g.) was dissolved in carbon tetrachloride (20 c.c.), an equal amount of iodine chloride solution was added and the contents were kept in the dark for 3 hours. The excess of iodine was then titrated in the usual way with sodium thiosulphate. The I. V. obtained was 57·4 and that required for one double bond in the above formula 54·2.

α-Calotropeol.—The acetate (2·0 g.) was dissolved in benzene (50 c.c.), an equal amount of 5% alcoholic potash was added to it and the mixture was boiled under reflux for 5 hours. The major bulk of the solvents was distilled off and the residue was diluted with water. The resulting solid was washed free of alkali and dried. It was dissolved in a mixture of boiling acetone (125 c.c.) and ether (20 c.c.) and allowed to crystallise slowly in the refrigerator. A good amount of crystalline solid was collected at the bottom of the flask and was recovered by filtration. It appeared as transparent rods and narrow plates under the microscope and melted at 204–5°. It resembled the acetate in its solubility in the ordinary organic solvents except benzene in which it was more sparingly soluble. It gave a bright pink solution immediately with the Liebermann-Burchard reagent; the colour changed to purple in about an hour; after 2 hours it was pale blue and slowly faded to yellowish brown (5 hours). These changes could be brought about rapidly by the addition of a few drops of water. With the Salkowski reagent the sulphuric acid layer was coloured orange-yellow exhibiting deep green fluorescence. [Found: C, 84·6; H, 11·4; $C_{30}H_{50}O$ requires C, 84·5; H, 11·7%]. $[\alpha]_D^{32°}$, + 102·0° in benzene solution.

Benzoate of α-Calotropeol.—The crystalline alcohol (0·6 g.) was dissolved in benzene (40 c.c.) and pyridine (8 c.c.) and benzoyl chloride (7 c.c.) were added to it. The mixture was set aside for 12 hours and then heated under reflux for 3 hours on a water-bath. The solvents were removed under low pressure and the resulting residue was dissolved in ether-benzene mixture (150 c.c.). The solution was washed with 1% sodium hydroxide solution followed by aqueous sulphuric acid. Finally it was washed free of acid with water. It was dried over anhydrous sodium sulphate and gently warmed to remove the ether as far as possible. To the concentrate (20 c.c.) an equal amount of alcohol was added and the mixture was allowed to cool slowly. A crystalline solid melting at 273–74° was obtained and no change in the melting point was effected by crystallising it from ligroin (20 c.c.). It crystallised in the form of broad rectangular plates. [Found: C, 83·6; H, 9·8; $C_{37}H_{54}O_2$ requires C, 83·8; H, 10·2%]. $[\alpha]_D$, + 74·3° in benzene solution. It gave the same colour reactions as the acetate.

Isolation of acetate of β-calotropeol and β-amyrin.—The ethyl acetate mother-liquor left after removing the acetate of α-calotropeol was concentrated and the earlier fractions which were mixtures of this compound were removed. Later a small fraction melting at 232–35° was obtained. Repeated recrystallisation of this from ethyl acetate yielded a product melting at 238–39° which proved to be identical with β-calotropeol acetate (see below). Though the existence of β-calotropeol in the resinol mixture was thus indicated, its isolation in larger quantities was conveniently effected by the benzoate method.

The final ethyl acetate filtrates from which the acetates of the calotropeols had been separated and the original ether and benzene mother-liquors were all united, evaporated to dryness and the residue carefully studied. It (4·0 g.) was dissolved in ether (150 c.c.), an equal amount of alcohol was added and the contents were stirred well. Immediately a solid melting at 205–8° and containing acetates of calotropeols separated out ; it was removed by filtration. The mother liquor was concentrated in stages and five fractions were collected. The first of these melted between 173–85° and the last 90–135°. The last two fractions were coloured yellow. By fractionation of these several times from acetone and ethyl acetate, a small amount of a solid melting at 190–206° was collected. Further crystallisation from benzene-alcohol mixture and finally ethylacetate yielded a product melting at 239–40° and crystallising in the form of long prismatic rods. It produced a pink solution with the Liebermann-Burchard reagent and no change in the colour was effected by the addition of water; with the Salkowski reagent a yellow solution was formed. It was different from the acetates of the calotropeols and the mixed melting points with these compounds were considerably depressed. From its properties it appeared to be β-amyrin acetate and this surmise was proved to be correct by a mixed melting point determination using an authentic sample of β-amyrin acetate obtained from the roots of the Decalepis. On hydrolysis it yielded β-amyrin.

Benzoate method (β-calotropeol benzoate).—Solid (B) (5·0 g.) was dissolved in benzene (30 c.c.), benzoyl chloride (12 c.c.) and pyridine (10 c.c.) were added and the contents were set aside for 12 hours. Then the mixture was heated on a water-bath for 5 hours. The solvents were removed completely under low pressure and the residue was taken up with water. The aqueous suspension was extracted with a mixture of ether and benzene (2 : 1), (300 c.c.) in small lots and the ether-benzene layer was washed with 1% aqueous sodium hydroxide, dilute sulphuric acid and water in succession. The solution on concentration to about 100 c.c. deposited a solid (3·0 g.) melting at 200°. It was digested with ether (100 c.c.) and the ether-insoluble

portion was carefully separated by decanting off the solution. On crystallisation from a mixture of benzene and alcohol (50 c.c.) it yielded a fraction (0·8 g.) melting at about 260°. Further purification of the solid was effected by dissolution in petrol (50 c.c.) and cooling the solution in ice after concentrating to half the bulk. As a result a solid (0·4 g.) melting at 274–77° separated out and after one more crystallisation from the same solvent it was obtained as long rectangular plates melting at 279–80°. No more rise in the melting point could be effected by further purification. With the resinol colour reagents it reacted to produce coloured solutions similar to those given by α-calotropeol and its derivatives. [Found: C, 83·7; H, 10·4; $C_{37}H_{54}O_2$ requires C, 83·8; H, 10·2%]. $[\alpha]_D^{33°}$, + 69·0° in benzene solution. The molecular weight determined as already described by finding the saponification equivalent was 538, the value required for the above formula being 530. The mixed melting point with α-calotropeolbenzoate was considerably depressed.

β-Calotropeol.—The benzoate (0·8 g.) was dissolved in benzene (45 c.c.) and the solution was boiled with an equal amount of N/2 alcoholic potash for 5 hours. The major bulk of the solvents was distilled off and the residue was treated with water. The resulting solid (β-calotropeol) was filtered and washed free of alkali. It melted at 216–17°, after a crystallisation from benzene-alcohol mixture (2 : 1). On concentrating the mother-liquor in stages, only the above substance could be obtained. The alcohol as well as the benzoate were thus shown to be pure. The mixed melting point with α-calotropeol was found to be depressed (175–95°).

β-Calotropeol appeared as prismatic rods under the microscope. It was practically insoluble in alcohol and readily dissolved in ether, chloroform and benzene. In the last solvent its solubility was less than that of its acetate (see below). It produced the same colour reactions with the Salkowski and the Liebermann-Burchard reagents as α-calotropeol. [Found: C, 84·7; H, 11·2; and $C_{30}H_{50}O$ requires C, 84·5; H, 11·7%.] $[\alpha]_D^{32°}$, + 50·9° in benzene solution. [Found: iodine value, 57·8; $C_{30}H_{50}O$ requires for one double bond, 59·6].

β-Calotropeol Acetate.—β-Calotropeol (0·5 g.) was dissolved in acetic anhydride (10 c.c.), a few drops of pyridine were added and the solution boiled for 3 hours. On adding water and allowing the contents to stand overnight the acetate separated out as a colourless solid. It was crystallised from ethyl acetate (20 c.c.) and the resulting crystalline compound melted at 238°. Under the microscope it appeared as elongated hexagonal plates. When crystallised from benzene-alcohol mixture, it was obtained as a woolly

crystalline mass and it appeared as thin rods under the microscope. It was easily soluble in benzene and chloroform, moderately soluble in ether and ethyl acetate and practically insoluble in ethyl and methyl alcohols. It produced the same colour reactions as the benzoate. The substance was unaffected when boiled with formic acid. [Found: C, 81·7; H, 10·9; and $C_{32}H_{52}O_2$ requires C, 82·1; H, 11·1%.] $[\alpha]_D^{30°}$, +43·9° in benzene solution. Mixed melting point with α-calotropeol acetate was found to be depressed (225–230°).

When the mother-liquors from the crystallisation of the benzoate mixture were worked up some more of the β-calotropeol benzoate could be obtained and it was not possible to isolate α-calotropeol or β-amyrin esters.

Solid (C) (4·0 g.) *(β-amyrin and tetracyclic compounds)*.—It was yellow in colour and sticky to the touch and was found to be soluble in all organic solvents making purification by crystallisation difficult. From its colour reactions it was considered to consist mostly of resinols. To facilitate the isolation of crystalline products it was studied by the acetate method. Its acetylation was carried out in the usual way by boiling the solid (4·0 g.) with acetic anhydride (15 c.c.) in presence of sodium acetate for 3 hours. The mixture was diluted with water and after allowing to stand for some hours it was ether extracted. On concentrating the ether solution to about 30 c.c. a crystalline solid mixed up with an yellow amorphous substance separated out. Repeated washing with small quantities of ether removed the coloured portion leaving behind colourless crystals melting at 218–30°. Two more crystallisations of the above fraction (1·5 g.) from ethyl acetate resulted in the separation of a colourless crystalline compound melting sharp at 240°. It was identified as β-amyrin acetate by making a mixed melting point determination with an authentic sample.

The ether mother-liquor and washings, on careful concentration in stages, deposited some more of crude amyrin acetate. The final solution was evaporated almost to dryness when a residue was obtained in the form of a thick syrup. It was shaken well with acetone (30 c.c.) and on allowing the contents to stand for about 6 hours a colourless solid melting at 148–60° was deposited. The mother-liquor on careful manipulation yielded some more of the above solid. On crystallisation from acetone it was obtained in the form of broken cubes and it melted at 170–74°. Further purification was not attempted since the yield of the substance was only 0·5 g. [Found: C, 82·6; H, 11·2; $C_{32}H_{52}O_2$ requires C, 82·1 and H, 11·1%]; $[\alpha]_D^{30°}$, +44·0° in benzene solution. It differed from β-amyrin acetate in producing a yellow solution exhibiting green fluorescence with Liebermann-Burchard

reagent in place of a pink coloured solution. This property was made use of in the above fractionation to detect and eliminate β-amyrin acetate which if present even in traces would invariably produce the pink solution. With the Salkowski reagent it produced a yellow solution with green fluorescence. The low melting point of the acetate coupled with the colour reactions indicated that it was probably a tetracyclic resinol mixture. This surmise was confirmed by effecting ring closure by the following two methods: Formic acid (20 c.c.) was added to the benzene solution (20 c.c.) of the substance and the mixture was boiled for 3 hours. The contents were largely diluted with water and the benzene layer was separated, washed free of acid and was concentrated to about 5 c.c. On the addition of an equal volume of alcohol and cooling the solution in ice, a crystalline solid melting at about 211–15° was obtained. This produced the characteristic colour reaction of the pentacyclic triterpenes, *i.e.*, pink solution with the Liebermann-Burchard reagent. However, the benzene-alcohol mother-liquor from the above compound yielded on evaporation a large amount of the unchanged original compound. This was dissolved in chloroform (15 c.c.), the solution was saturated with dry hydrogen chloride at 0° C. and kept at that temperature overnight. The residue obtained after removing the solvent was crystallised from benzene-alcohol and ethylacetate in succession resulting in the isolation of the transformation product in a better yield.

The final mother-liquors left after removing the crude tetracyclic compounds were examined in detail to see if any sterol-like substances could be detected. No fraction gave the proper sterol colour reactions when tested with the Liebermann-Burchard and Salkowski reagents. Solid (C) thus consisted mostly of β-amyrin and small quantities of tetracyclic compounds.

Filtrate (II) (Cardiac poisons and calcium oxalate).—The filtrate obtained from the coagulum (I) on cooling in the refrigerator deposited only a small amount of a slimy solid. Hence the whole solution was extracted repeatedly with ether employing 500 c.c. of it at a time. The ether solutions were united and extracted with 5% aqueous sodium carbonate twice. The ether layer was washed free of alkali and dried over anhydrous sodium sulphate; on distilling off the solvent it left behind a residue melting at about 235°. It was further purified by dissolving in chloroform (100 c.c.) and reprecipitating by the addition of petrol (500 c.c.). The purified solid (0·4 g.) was obtained in the form of colourless rods and melted at 241°–43°. It produced a green solution with the Liebermann-Burchard reagent and the colour of the solution changed to red after a day. It dissolved in strong hydrochloric acid producing a greenish blue solution. It tasted bitter leaving a tingling sensation on the tongue.

The carbonate solution was acidified with hydrochloric acid and was slightly warmed, when an offensive smell emanated. The acid solution was repeatedly ether extracted and all the ether solutions were united. The solvent was completely removed by distillation and the residue was dissolved in chloroform and reprecipitated by the addition of petroleum ether. The resulting product was very small and was not further studied.

The original filtrate (II) which had been extracted with ether, was extracted with chloroform in four lots employing 400 c.c. of chloroform each time. All the chloroform solutions were mixed and the solution was concentrated to about 25 c.c. No solid separated out even on prolonged cooling in the refrigerator. Then about 100 c.c. of petrol were added and the contents were well stirred when a solid slowly began to separate out. It was crisp and it melted at about 220° (yield 2·0 g.). It gave colour reactions very similar to those of the compound melting at 241°. They were both toxic to fish and in general resembled usharin and related compounds isolated by Hesse *et al.* from the mixed latex obtained from *Calotropis procera* and *gigantea.* No further detailed study was made of them.

After the filtrate (II) had been extracted with chloroform and the chloroform layer removed, a small quantity of an insoluble compound slowly began to separate out from the aqueous layer. It was found to be insoluble in alcohol also. Making use of this property, the substance could be completely separated from the aqueous solution by adding a liberal quantity of alcohol (600 c.c.) and leaving the contents undisturbed for a week. A sticky solid thus precipitated down at the bottom of the flask and was filtered at the pump. Further purification was effected by digestion with alcohol (500 c.c.) whereby it was obtained as a non-sticky solid (6·0 g). It neither melted nor burnt when introduced into a flame and hence was considered to be an inorganic salt. Further it was not soluble in any organic solvent or in water. In acetic acid medium it appeared as plates and bunches of needles under the microscope. Its resemblance in crystalline structure to an authentic sample of calcium oxalate was very marked. Further on ignition calcium oxide was obtained in quantitative yield. It readily dissolved in hot dilute sulphuric acid and the solution reduced potassium permanganata. From the above properties it was identified as calcium oxalate and its estimation was carried out by permanganate titration. Most of the solid (95%) was found to be calcium oxalate, the yield being 1% on the weight of the latex.

The aqueous alcoholic solution was concentrated to about 500 c.c. and was treated with hot neutral and basic lead acetate solutions in succession. The insoluble lead salts were collected and decomposed separately in the

usual way by passing hydrogen sulphide through the aqueous suspensions. After removing the lead sulphide the filtrates were extracted with ether and chloroform in succession. From the ether solution only a small quantity of a crystalline resin acid melting at 135° could be obtained. From the chloroform solution no compound could be obtained; all the chloroform-soluble substances had obviously been removed earlier. The aqueous filtrate on further concentration and hydrolysis with aqueous sulphuric acid yielded amorphous resins.

Summary

The latex of *Calotropis gigantea* contains (1) resinols as esters of steam-volatile fatty acids (acetic and isovaleric), (2) cardiac poisons similar to usharin and (3) calcium oxalate. The resinol portion consists mainly of two new alcohols, α-calotropeol and β-calotropeol in almost equal quantities and minor amounts of β-amyrin. The important properties of the calotropeols have been studied. It is suggested that 'Calosterol' of Basu and Nath should be a mixture of resinols contaminated with cardiac poisons. The recent report of Hesse *et al.* that the milky latex of *Calotropis procera* contains only α-lactucerol as its esters with steam-volatile fatty acids is discussed.

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