CHEMICAL EXAMINATION OF THE FLOWERS OF *PONGAMIA GLABRA* AND A NOTE ON THE GLYCOSIDIC COMPONENTS OF *BUTEA FRONDOSA* FLOWERS

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In the past the seeds and the roots of *Pongamia glabra* have been studied in detail. The former contains a fixed oil, some waxy matter consisting mostly of-long chain fatty acids and three crystalline substances, karanjin, pongamol and glabrin. Karanjin has been investigated in detail, its constitution established and synthesis effected. The other two are still under study.[1] The root bark yields a new crystalline substance named kanugin whose constitution is again unsettled.[2] The flowers of this plant have also been recorded to be of medicinal value. An attempt has now been made to make a complete analysis of this plant material, to isolate crystalline substances, and identify or characterise the components.

The flowers of *Pongamia glabra* are small in size and they fall down in large quantities in the months of January and February. This is the main season and there is another flowering later on when smaller quantities could be obtained. When fresh, the colour is ivory white and it turns yellowish brown on drying. In a preliminary examination a small quantity of the dry flowers was extracted suscessively in a Soxhlet extractor with different solvents and each extract studied. For a large-scale examination ligroin, ether and alcohol alone were used. The ligroin extract was separated into several fractions using alcohol. The main component was wax which was found to contain about 12% of hydrocarbons and the remainder was made up of esters derived from alcohols of C_{24} to C_{34} dimensions and fatty acids with chain lengths C_{24} to C_{30}. A small amount of oil resembling in its characteristics the fixed oil from the seeds was also present. Besides these a small quantity of a colourless crystalline substance melting at 212° was isolated from the ligroin fraction. It does not give the reactions for sterols or resinols, but seems to resemble in a way karanjin and the other crystalline principles isolated from the seeds and roots. It is only feebly bitter and feebly toxic to fish. Its composition may be represented by the formula $C_{15}H_{12}O_5$; it is named ' pongamin '.

The ether extract of the ligroin extracted residue was a coloured solid from which could be isolated two crystalline substances. The first

was a yellow substance which answered all tests for kæmpferol; it occurred free. The second was a colourless solid obtained after saponifying waxy matter and was identified as γ-sitosterol.

The final extraction with alcohol was made in two stages, first using alcohol of about 90% strength and subsequently 80% alcohol. This procedure was already employed in connection with the roots of *Decalepis Hamiltonii.*[3] From the extract a sparingly soluble fraction crystallised out. This could be again separated into two parts using their solubility in water. The water insoluble part was found to be a phytosterolin agreeing in all its properties with γ-sitosterol monoglucoside. The same substance was also isolated from the flowers of *Butea frondosa,* another leguminous plant. The water-soluble portion was a sweet-tasting nitrogenous substance closely resembling glabrin[1] in most of its properties. It however differs in having a different composition and in having a lower rotation. It is therefore named neoglabrin.

The more soluble portion of the alcohol extract contained a saponin which is named glabrosaponin. It seems to have the composition $C_{50}H_{84}O_{23}$ and the aglucone seems to have the formula $C_{30}H_{50}O_5$. The sugar portion is rather complex and yields an indefinitely melting osazone mixture. A number of triterpene saponins isolated from plants, are known to contain three or four different sugar groups in their molecules. But their constitutions are still uncertain. It may be recalled here that a similar though not identical saponin was found to be present in the flowers of *Butea frondosa.*

Of the different parts of the tree so far examined the flowers provide the largest number of extractable components. Besides wax and oil, they contain pongamin, kæmpferol, neoglabrin, phytosterolin and glabrosaponin; the last two are not found even in the seeds. Waxy matter is present in the maximum amount in the flowers. Kæmpferol has already been found in plants of the family Leguminoseæ (senna, indigo, etc.) and hence its occurrence in the flowers of the pongamia is not unusual; but it is remarkable that it occurs entirely free and not as glycoside.

There exists a general resemblance between the components of these flowers and of the flowers of *Butea frondosa* though in details differences exist. Besides colouring matter they both contain resinol-glycosides (saponins), sterolin (the same substance, γ-sitosterol glucoside) and wax. The colouring matter is different, in one case kæmpferol is a flavonol and in the other case it is butin, belonging to the flavanone group, and its derivatives. Pongamia wax contains some hydrocarbons whereas the Butea flower wax is composed almost completely of esters.[4]

Experimental

The fresh flowers were collected in the month of February from the trees of the Andhra University forest area in Waltair, dried quickly in the sun and preserved in covered tins for subsequent use. They were ground into a coarse powder and employed in the following experiments.

100 grams of the dry material were extracted in a Soxhlet extractor successively with several solvents. The yields and the general characteristics of the various extracts are given below.

Solvent	Yield %	Nature of the Extract
1. Ligroin	0·9	Pale brown semi-solid consisting of waxy and oily matter
2. Ether	0·5	Dark green semi-solid ; contained a yellow crystalline substance having properties of hydroxy-flavones
3. Acetone	0·2	Yellow waxy matter
4. Ethyl alcohol	1·0	An almost colourless solid partly soluble in water

As already mentioned in the Introduction, when working with large quantities for purposes of detailed study, only ligroin, ether and alcohol were used as solvents and acetone was omitted since it did not extract much of any distinctive material.

Ligroin Extract

The dry flower powder (10·0 *kg.*) was extracted with ligroin (b.p. 80–110°) in a continuous extractor in batches, each batch being extracted for 20 hours. The major bulk of the solvents was then recovered by ordinary distillation and the last traces were removed under reduced pressure. The residue (90·0 g.) thus obtained was semi-solid in consistency and light brown in colour and had a faint aromatic odour. It was digested under reflux with boiling alcohol (500 c.c.) for 15 minutes, the supernatant solution was poured off and the residual heavy liquid again extracted four times in a similar manner. The insoluble portion gradually solidified on cooling and was marked (A) (40·0 g.). The collected alcoholic solution was concentrated to half the bulk and allowed to stand. The pale yellow solid obtained thereby was designated (B) (19·0 g.) and the final mother liquor (C).

Fraction A: Wax

The brown insoluble solid (A) melted at about 60° and after one crystallisation from benzene-alcohol mixture (1 : 1) it was pale yellow in colour and melted at 68–75°. It was subjected to saponification as given below:

The solid (35·0 g.) was dissolved in benzene (600 c.c.), to the solution 8% alcoholic potash (100 c.c.) was added and the contents were boiled under reflux for 20 hours. Then the major bulk of the solvents was distilled off, to the concentrate purified pumice stone was added and the mixture was quickly dried to a friable mass. It was extracted in a Soxhlet, avoiding access of moisture, with dry acetone for about 20 hours, immediately followed by further extraction with dry ether for 6 hours. Both the acetone and the ether extracts were united and the solvents were removed. The crude unsaponifiable matter thus obtained was again subjected to saponification by boiling its benzene solution (300 c.c.) with 7% alcoholic potash for 8 hours. The solvents were distilled off, water was added to the residue and the aqueous suspension was subjected to repeated ether extraction. All the ether extracts were united and washed free of alkali with water. The ether solution was dried over anhydrous sodium sulphate and distilled to remove the solvent completely.

The unsaponifiable matter (20·5 g.) was brown in colour and melted indefinitely below 65°. After one crystallisation from a mixture of acetone and ether it lost most of its colour and melted between 66-75°. It was then dissolved in cold ligroin and set aside for several hours. A crystalline solid separated melting at 79–81° (10·0 g.). After three crystallisations from acetone it melted sharp at 87° and solidified at 86°. (Found: C, 82·4; H, 14·3; $C_{30}H_{62}O$ requires C, 82·2; H, 14·1%.) The acetate obtained by heating it with acetic anhydride and sodium acetate crystallised from alcohol as colourless needles and melted at 72°. Therefore this fraction sparingly soluble in ligroin consisted mostly of long-chain alcohols corresponding to the description of myricyl alcohol which has been shown by Chibnall *et al.*[5] to be a mixture of the following composition: 40% of C_{30}, 40% of C_{32}, and 20% of C_{34} alcohols.

The ligroin soluble fraction (10·0 g.) was obtained by removing the solvent. It was dissolved in boiling amyl alcohol (100 c.c.), treated with an equal volume of concentrated hydrochloric acid, the mixture boiled for about 5 minutes and then allowed to cool slowly. When the temperature reached 60° a solid cake was formed at the top; it was removed and the above treatment repeated. An almost colourless solid consisting essentially of hydrocarbons resulted (7·0 g.). In order to remove the last traces of alcohols, it was dried and boiled with acetic anhydride (15 c.c.) and sodium acetate (6·0 g.) for 3 hours, the mixture was treated with a large amount of water and kept in the ice-chest. The resulting solid was treated with alcohol and acetone in succession in order to remove soluble acetates. The product (6·5 g.) was free from alcohols. (Found: C, 85·0; H, 14·5%.) It

was then dissolved in cold petroleum ether (200 c.c.) and the clear solution allowed to concentrate slowly. Six fractions were thus collected having melting points between 57° and 68°. Pairs of neighbouring fractions were united and carefully recrystallised and thus three sharp-melting solids were obtained. They were subjected to final treatment with sulphuric acid at 130° according to the method of Piper *et al.*[6] Their characteristics are given below; the melting and setting points were obtained by slow heating and cooling.

M.P.	S.P.	Analysis	Average chain length	Probable composition
68·5°	68·0°	C, 85·3 ; H, 14·6%	$C_{31\cdot4}$	20% C_{33}+80% C_{31}
63·0°	62·5°	C, 85·4 ; H, 14·4%	$C_{28\cdot8}$	95% C_{29}+5 % C_{27}
59·0°	58·5°	C, 85·2 ; H, 14·7%	$C_{27\cdot8}$	C_{27} mostly

The average chain lengths of the mixtures were obtained by reference to the curves given by Piper *et al.*[6] The probable composition of the mixtures is given only roughly by referring to the melting and setting points given by Chibnall *et al.*[5] for known mixtures of synthetic paraffins.

The soap left after the removal of the unsaponifiables was decomposed by heating with hydrochloric acid and the solid separating on the top was isolated. This was taken up in ether and washed free of mineral matter. Then the ether solution was distilled to remove the solvent; the residue (14·0 g.) melted at about 70°. It was crystallised repeatedly first from ethyl acetate and then finally from alcohol. Three fractions having sharp melting points were obtained. The average chain lengths were calculated from the molecular weights and the pobable composition of the mixture obtained by referring to the tables given by Piper *et al.*[7] relating to the melting points of known acid mixtures using synthetic compounds. Their properties and probable compositions are given below:

	Melting point	Molecular weight by titration	Average chain length	Probable composition
1.	84·0°	410·8	C_{27}	20% C_{30}, 40% C_{28} and 40% C_{26} acids
2.	79·0°	380·6	C_{25}	20% C_{28}, 40% C_{26} and 40% C_{24} acids
3.	74·0°	350·2	C_{23}	C_{24} and immediate homologues

Fraction B: Wax

It (19·0 g.) was divided into two fractions by crystallising from alcohol, benzene-alcohol mixture and finally from ether. The less soluble

fractions (5·0 g.) melted at about 86° (B_1) and the more soluble one (B_2) melted much lower (10·0 g.). Each of them was saponified separately. (B_1) yielded an alcohol mixture (2·2 g.) melting sharp at 87° agreeing in all respects with the C_{30} alcohol mixture (myricyl alcohol) obtained in fraction (A). (Found: C, 82·1; H, 14·0; $C_{30}H_{62}O$ requires C, 82·2, H, 14·1%). The acetate crystallised as colourless needles melting at 72° (Found: C, 79·8; H, 13·6. $C_{32}H_{64}O_2$ requires C, 80·0 and H, 13·3%.) The acid fraction (2·4 g.) melted at 87° and had a molecular weight of 440·4. It therefore had an average chain length of C_{29} and was probably a mixture of C_{32}, C_{30} and C_{28} acids.

(B_2) yielded a nonsaponifiable portion part of which was a steam volatile liquid. The non-volatile portion (3·5 g.) when purified yielded a mixture of wax alcohols melting at 72–76° (Found: C, 82·1, H, 13·8%). It therefore consisted of C_{24} and C_{26} alcohols mostly. The fatty acid mixture (4·0 g.) contained some liquid also; it was separated by the lead salt method. The liquid acids constituted about 12%; the solid acids, when crystallised, melted at 78° and had the mean molecular weight of 375·2. This corresponded to a mixture of C_{26} and C_{24} acids.

Fraction C: *Oil, etc.*

When the alcoholic solution was concentrated to about 250 c.c. some wax separated and it was removed. On distilling the solvent completely a brown oil was obtained (38·5 g.). This was dissolved in ether (100 c.c.) and allowed to stand overnight in a refrigerator. A yellow solid (D) was deposited. It was filtered and washed with a little ether. The solution was dried over anhydrous sodium sulphate and the solvent removed under reduced pressure. The pale brown oil had the following properties: Sp. gravity 0·9321; ref. index 1·4726; acid value 39·8; sap. value 186·6; iodine value 79·2. It thus resembled the oil from the pongamia seeds[8]; no further examination was made.

Solid (D): Pongamin

The crude solid was recrystallised twice from boiling alcohol whereby a colourless substance (0·5 g.) was obtained in the form of long flat needles. It melted at 210–12° (decomp.). It was insoluble in water and only sparingly soluble in ether. Its solution in alcohol did not produce any colour with ferric chloride or sodium hydroxide. It dissolved in concentrated sulphuric acid to produce a yellow solution which changed to green on standing (resemblance to karanjin). Its alcoholic solution produced only a yellow colour on reduction with magnesium and hydrochloric acid and no colour by reduction with sodium amalgam. It did not give the characteristic

reactions of rotenone. (Found: C, 66·5; H, 4·8; $C_{15}H_{12}O_5$ requires C, 66·2; H, 4·4%.) The molecular weight by Rast's micromethod varied between 230 and 242; the value required for the above formula is 272.

Ether Extract: *Kæmpferol*

This was obtained as a greenish semi-solid (40·0 g.) on removing the solvent. It was boiled with alcohol (400 c.c.), the insoluble waxy matter was separated and the filtrate gradually concentrated to about a third of its bulk, the amorphous impurities that separated meanwhile being collected separately. It was then further concentrated to a syrupy consistency and allowed to crystallise. The product now melted at 252°–55°. Further crystallisation from dilute alcohol yielded a yellow needle-shaped crystalline solid melting at 274°–75° (decomp.). The yield of the pure substance was 4·0 g. Its alcohol solution gave an olive brown colour with ferric chloride and a deep yellow one with alkali. On reduction with magnesium and hydrochloric acid it developed the characteristic anthocyanin colour, deep pink. It dissolved in strong sulphuric acid exhibiting a powerful bluish green fluorescence. (Found in an air-dried sample: C, 59·5; H, 4·3 and $C_{15}H_{10}O_6$, H_2O requires C, 59·2 and H, 3·9%. Loss on drying at 120° for 3 hours *in vacuo*: 6·2%; $C_{15}H_{10}O_6$, H_2O requires for one H_2O, 5·9%.)

By acetylation using sodium acetate and acetic anhydride, a colourless acetate was obtained which crystallised from alcohol in the shape of needles. On heating it sintered and shrank markedly at 118°–20° and finally melted at 181°–182°. (Found in air-dried sample: C, 58·2; H, 3·8; $C_{23}H_{18}O_{10}$, H_2O requires C, 58·5 and H, 4·2%. Loss on drying at 110° for 2½ hours *in vacuo*, 3·6%; $C_{23}H_{18}O_{10}$, H_2O requires for one H_2O, 3·8%. Found in the sample dried at 110°: C, 60·4; H, 4·3 and $C_{23}H_{18}O_{10}$ requires C, 60·8 and H, 4·0%).

The above colour reactions and the analysis indicated that the pigment was kæmferol. This was confirmed by comparison of the substance and the acetate with authentic samples obtained from senna leaves. No depression in mixed melting points was noticed.

Though the dual melting point of the acetate was noticed long ago, there has been difference of opinion regarding the cause of it. Perkin and Wilkinson[9] believed that it was not due to solvent of crystallisation. Tutin[9] seemed to have studied the matter more closely. Employing samples crystallised from various solvents he noted that they underwent some loss when dried at 110° and the dried samples melted sharp at 183° without any previous sintering. He, therefore, concluded that some solvent of crystallisation was involved. Though he did not analyse air-dried samples for carbon and

hydrogen, the loss on drying of a sample of the acetate crystallised from ethyl acetate corresponded to the presence of half a molecule of ethyl acetate of crystallisation. From the results (C and H values) presented above it is definite that the substance crystallises from ordinary alcohol with one molecule of water of crystallisation which it loses at 110°. It is interesting to note that even kæmpferol comes as a monohydrate. In general acetates of hydroxy-flavones and -flavonols are found to be anhydrous. Kæmpferol acetate seems to be therefore an exception.

The alcohol insoluble and coloured waxy matter (8·0 g.) was dissolved in ether (500 c.c.) and extracted twice with aqueous sodium carbonate (200 c.c.) resulting in the removal of some dark slimy matter. Further extraction with 5% sodium hydroxide (6 times with 50 c.c. each time) yielded a coloured, deep red extract. From this after acidification and ether extraction some more of kæmpferol (0·8 g.) was obtained.

Sterol

The alkali-washed ether solution was distilled and the residue (5·0 g.) was found to consist of some waxy substance. It was hydrolysed by boiling its benzene solution with alcoholic potash for 7 hours and the non-saponifiable matter was separated by ether extraction. By repeated crystallisation a small quantity of a sterol fraction was obtained. This was acetylated by boiling with acetic anhydride and sodium acetate and the resulting product was crystallised from alcohol. It appeared as needles and rods and melted at 138–39°. On hydrolysis it yielded a sterol which on repeated crystallisation from alcohol melted at 146°. This was found to be identical with the sitosterol obtained from the phytosterolin described later.

Alcohol Extract

The flower powder left after ether extraction was extracted with methylated spirits (90%) in the continuous extractor for about 15 hours. By this time all the soluble components were removed; then 10% water was added to the solvent and the extraction was continued in the same apparatus for 5 more hours. By this process components that are more readily soluble in aqueous alcohol were also extracted completely. The resulting aqueous alcoholic extract was concentrated to about 800 c.c. and set aside for a week. Glistening crystals embedded in resin separated out. This was filtered and the solid (E) and the filtrate (F) were studied separately.

Fraction E : Phytosterolin

The solid E was washed with ether in order to remove soluble impurities and then digested with water. A portion of it dissolved forming a brown

solution. The residue was insoluble in ether, alcohol and acetone but it dissolved almost completely in boiling acetic acid or pyridine. After two crystallisations from acetic acid, it reached a constant melting point 261–62° (decomp). The yield of the pure substance was 1·2 g. With Salkowski's reagent, the chloroform layer was coloured deep red and the sulphuric acid layer exhibited a powerful green fluorescence. With the Liebermann-Burchard reagent, a pink colour was immediately produced and it rapidly changed to blue and green. (Found: C, 72·0; H, 10·6 and $C_{34}H_{58}O_6$ requires C, 72·6; H, 10·3%; $C_{35}H_{60}O_6$ requires C, 72·9, H, 10·4%.)

The sterolin was hydrolysed by boiling with amyl alcoholic hydrochloric acid and the solvent was removed by steam distillation. The residue was ether extracted and the ether solution was distilled to remove the solvent. The product was crystallised from alcohol when a crystalline sterol (needles and plates) melting at 145-46° was obtained; it formed an insoluble digitonide. (Found: C, 83·8: H, 12·3 and $C_{28}H_{48}O$ requires C, 84·0; H, 12·0%; $C_{29}H_{50}O$ requires C, 84·1; H, 12·1%.) The acetate was prepared in the usual way by boiling with acetic anhydride and sodium acetate. It crystallised as needles and rods from alcohol and melted at 138–39°.

The aqueous solution gave tests for the presence of a reducing sugar and it was identified as glucose by the preparation of the osazone. From the properties of the sterol and its acetate it appeared to be γ-sitosterol. Hence the substance melting at 261–62° may be considered to be γ-sitosterol glucoside. These three compounds were found to be identical with the sterolin, the sterol and its acetate obtained from the flowers of *Butea frondosa* and there was no depression in the melting points.

Neoglabrin

The aqueous solution obtained from (E) was decolourised with 'norit' and concentrated to about 300 c.c. on a water-bath. It was then largely diluted with alcohol (2000 c.c.) with stirring when a copious white precipitate slowly separated out. The contents were cooled in the refrigerator for 2 hours and then filtered. A shining colourless product was left on the filter and it appeared as narrow plates and rods under the microscope. It became dark red when heated to 240° and melted at 281–82° with decomposition. The yield was 0·5% on the weight of the flowers taken. It was insoluble in all solvents except water in which it readily dissolved even in the cold. It did not produce any coloured solutions either with alkali or with acid. It tasted sweet and did not have any toxic effect on fish. When boiled with acetic anhydride and sodium acetate it was completely resinified. It did not respond to Molisch's test for carbohydrates. It

contained nitrogen in addition to oxygen and hydrogen. (Found in air-dried sample: C, 38·9; H, 7·7; N, 9·9%; $C_9H_{20}N_2O_8$ requires C, 38·1; H, 7·0 and N, 9·9%. Loss on drying at 120° for 3 hours, 4·0%.) The substance was leavoratatory $(\alpha)_D^{20}$, −40·0° in water. It gave a blue copper salt and a positive nin-hydrin reaction. Thus it seemed to be an amino-acid closely allied to glabrin.[1]

Filtrate F: Glabrosaponin

The filtrate (F) was concentrated to half its bulk and allowed to stand in the ice-chest. Since no solid separated, it was treated with excess of ether so that it was saturated and a layer of ether was formed. After 2 days a brown semi-solid mixed with some crystalline matter separated. It was filtered using fluted filters and washed first with ether and then with water. Most of the colour was thus removed. The residue (20·0 g.) was then boiled with alcohol (300 c.c.) and enough water (about 100 c.c.) added so as to obtain a clear solution. On cooling the solution a colourless crystalline solid (shining hexagonal plates) separated out. It was filtered; some more quantity was obtained by concentrating the filtrate and saturating it with ether. It melted at 248–50° with decomposition. It did not dissolve completely in water but gave a turbid colloidal solution. On the addition of a small quantity of alcohol it formed a true solution. It was insoluble in ether, benzene, chloroform, etc. Its alcoholic solution did not produce any colour either with alkali or with ferric chloride. No colour was produced when it was reduced with magnesium and hydrochloric acid. It dissolved in strong sulphuric acid producing an orange-red solution changing to purple on standing. With the Liebermann-Burchard reagent it produced a purple solution. It was non-toxic to fish. There was no loss on drying the substance at 120° *in vacuo* for 3 hours. (Found in the sample dried at 120°: C, 57·2; H, 8·5 and $C_{50}H_{84}O_{23}$ requires C, 57·1, and H, 8·0%.) $(\alpha)_D^{30}$, + 22·4° in aqueous alcohol.

Hydrolysis of the Glycoside: Glabrosapogenin

The crystalline solid (2·0 g.) was taken in boiling 90% alcohol (400 c.c.) and strong sulphuric acid (40 c.c.) was slowly added. The clear solution was boiled under reflux for 7 hours. Then it was largely diluted with water and boiled to remove the major bulk of the alcohol. A colourless solid soon began to separate out and the contents were kept in the refrigerator overnight. A copious crystalline solid was thus obtained and it was filtered and washed free of acid. The crude product was dissolved in alcohol (100 c.c.) and set aside. The resulting small crop of crystals melted at 193° and was removed by filtration. The mother liquor on concentration and standing

deposited a purer solid in larger amounts melting at 195–96° (needles and rods under the microscope). It did not dissolve in water but dissolved readily in boiling alcohol. It did not respond to any of the well-known sterol colour reactions but gave the characteristic colour reactions of resinols. Thus it produced a yellow solution with sulphuric acid exhibiting green fluorescence and a purple solution with the Liebermann-Burchard reagent. No colour was produced either with alkali or with ferric chloride; it did not form a precipitate with digitonin (Found: C, 72·8%; H, 10·9; $C_{30}H_{50}O_5$ requires C, 73·5 and H, 10·2%.) $(\alpha)_D^{30}$, +72·3° in alcohol.

The aqueous acid solution obtained after hydrolysis was found to contain reducing sugars. From one portion of the solution sulphuric acid was removed by means of barium carbonate and the osazone was prepared by treatment with phenyl hydrozine hydrochloride and sodium acetate. Though it was obtained in the form of yellow needles, it melted indefinitely at about 180°. Another portion of the acid solution was carefully neutralised with aqueous sodium hydroxide and titrated against Fehling's solution. The amount of reducing sugars calculated as glucose was found to be 54·2% on the weight of the glycoside taken. The mother liquors left after separation of the saponins gave tests for the presence of small amounts of tannin.

A Note on the Glycosidic Components of Butea frondosa Flowers

The presence of two colourless glycosidic components in small quantities was recorded in a paper by Murti and Seshadri.[10] These have been studied in greater detail now. The dry flower petals were first extracted with ligroin in order to remove waxy matter. The fat and wax-free material was extracted with alcohol. This extract was concentrated as far as possible and treated with excess of water; a clear orange yellow solution was obtained (aqueous solution A). It was repeatedly shaken with ether in order to remove all ether-soluble portion.

Phytosterolin

When the ether extract was shaken with aqueous sodium bicarbonate in order to dissolve the butein-butin mixture present in it, a colourless solid separated out being insoluble both in the ether and aqueous layers. It was sparingly soluble in most solvents but could be recrystallised from glacial acetic acid or pyridine. A carefully purified sample has now been found to melt at 261–62° with decomposition. This gives the typical sterol reactions with Salkowski and Liebermann-Burchard reagents. (Found: C, 72·2; H, 10·6; $C_{34}H_{58}O_6$ requires C, 72·6; H, 10·3%; $C_{35}H_{60}O_6$ requires C, 72·9; H, 10·4%.)

The substance was hydrolysed with amyl alcoholic hydrochloric acid and the alcohol removed by steam distillation. By ether extracting the residual aqueous mixture was obtained a solid which was more easily soluble in most solvents and when finally crystallised from alcohol it melted at 145–46°. It gave the usual sterol colour reactions and formed a sparingly soluble digitonide; its acetate melted at 138–39°. The sterolin, the sterol and its acetate agreed in every respect with similar products obtained from the flowers of *Pongamia glabra*. As another product of hydrolysis the presence of glucose was established in the aqueous solution. Hence the sterolin was considered to be a glucoside of γ-sitosterol.

Saponin

Aqueous solution (A) that had been ether extracted was allowed to stand saturated with ether. The first crop of crystals that could be separated in the course of the first few days was pale yellow in colour and was different from butrin which separated later on. The pale yellow solid was repeatedly washed with warm water and recrystallised several times with dilute alcohol. It was then insoluble in ether and chloroform, more soluble in hot alcohol and readily soluble in pyridine and acetic acid. When recrystallised from dilute alcohol, it appeared as colourless hexagonal plates and melted at 237–38° with decomposition. Its colour reactions with Salkowski and Liebermann-Burchard reagents indicated that it belonged to the resinol group. As characteristic of all saponins it produced froth with water. (Found in a sample dried at 120°: C, 57·3; H, 8·4; $C_{50}H_{84}O_{23}$ requires C, 57·1; H, 8·0%.) In the analysis for carbon and hydrogen it resembles the saponin obtained from the flowers of *Pongamia glabra*. But the melting point of the Butea saponin is lower. This was hydrolysed by boiling with aqueous alcoholic sulphuric acid having concentration of 7% acid. After evaporating the alcohol a colourless solid was obtained. It was more easily soluble in ordinary solvents than the glycoside and on recrystallisation from alcohol it melted at 220°–22°. It gave all colour reactions of resinols and did not form a precipitate with digitonin in alcoholic solution. An air-dried sample lost 5·15% of its weight when dried at 120° for 2 hours. (Found in the oven dried sample: C, 74·9; H, 10·8; $C_{30}H_{50}O_4$ requires C, 75·9; H, 10·5%.)

Reducing sugars could be identified as other products of hydrolysis in the aqueous solution. No further data could be collected since the yield of the saponin was very small.

Summary

The flowers of *Pongamia glabra* have been examined in detail. The ligroin extract is made up of (1) aliphatic waxy matter, (2) some oil and

(3) a small amount of pongamin. The aliphatic waxy portion consists mostly of esters derived from C_{24} to C_{34} alcohols and C_{24} to C_{30} acids and smaller amounts (12%) of hydrocarbons of C_{27} to C_{33} dimensions. Pongamin is a colourless crystalline substance melting at 212° and having the approximate formula, $C_{15}H_{12}O_5$. It exhibits resemblance in properties to karanjin. The ether extract contains plenty of free kæmpferol and a small amount of γ-sitosterol occurring probably as an ester.

The alcohol extract contains small amounts of a sterolin melting at 262° and found to be γ-sitosterol glucoside. Larger amounts of neoglabrin and glabrosaponin have been found. The former is a high melting complex amino acid which is sweet in taste and resembles glabrin to a considerable extent. The glabrosaponin seems to have the formula $C_{50}H_{84}O_{23}$; it is a complex glycoside of a triterpenoid sapogenin having the probable formula, $C_{30}H_{50}O_5$.

New data are presented regarding the glycosidic components of the flowers of *Butea frondosa.* The sterolin is found to be the same as is present in the pongamia flowers. Though the butea saponin is similar being a triterpene glycoside it is not identical with glabrosaponin, neither are the aglycones the same.

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