

CONSTITUTION OF KANUGIN—PART II

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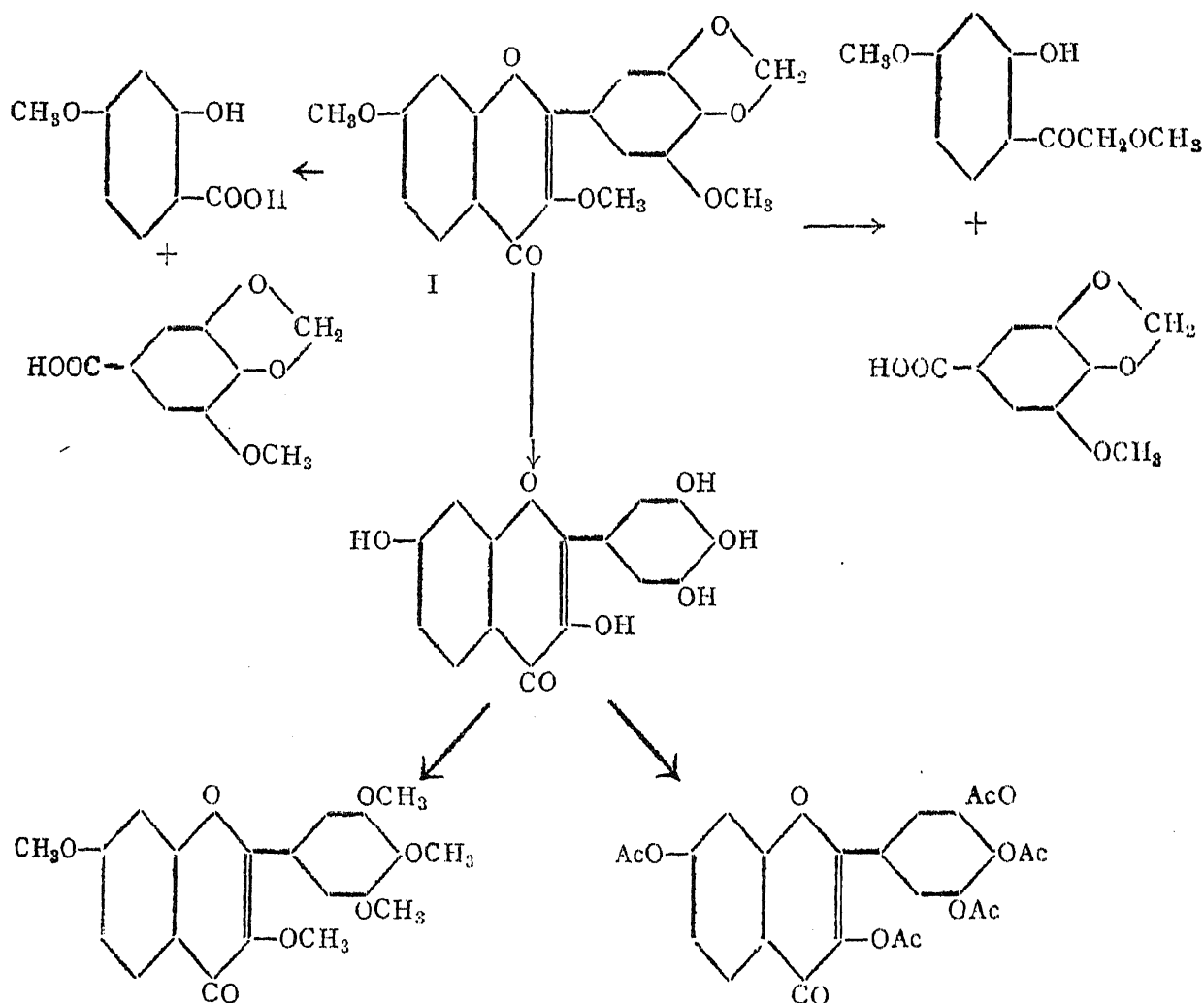
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IN Part I¹ were described certain earlier experiments which could not be continued due to the war and the dislocation of our laboratories. It was, however, shown that kanugin is a flavone derivative giving the characteristic colour reactions and that it has a resorcinol unit since it yielded *p*-methoxy-salicylic acid on oxidation with permanganate or decomposition with alkali. The reaction with hydriodic acid could not be explained as simple demethylation since nor-kanugin did not regenerate kanugin on re-methylation but yielded a different substance. Further work has now been possible and particulars regarding the complete constitution of kanugin are described in this paper.

Subsequent to our last publication it has been found that in the root bark of *Pongamia glabra* kanugin is accompanied by another related substance and careful and repeated crystallisation is necessary in order to remove it and obtain kanugin quite pure. The second component seems to be more in abundance in thicker and more mature roots that have been used in later work. Consequent on this thorough purification, the melting point of kanugin has been raised to 203–05° and its analysis is found to agree more closely with the formula $C_{19}H_{16}O_7$. Further with concentrated sulphuric acid and a crystal of gallic acid it gives a bright green colour characteristic of a methylene-dioxy grouping.

Degradation with alkali has been investigated in detail. A repetition of the fission of kanugin with aqueous potash has yielded two acid products which could be separated by means of their marked difference in solubility in water. The less soluble one is identified as myristicic acid by analysis, colour reaction, and by comparison with an authentic sample prepared starting from oil of nutmeg. The more soluble portion is found to be the same as the impure form of *p*-methoxy-salicylic acid mentioned in Part I. This identification has been confirmed by the conversion of the product into the 5-bromo-derivative and subsequent esterification to form the methyl ester of 5-bromo-4-methoxy-salicylic acid. These derivatives could be easily obtained pure since they are sparingly soluble, and they are found to be identical with authentic samples prepared from synthetic 4-methoxy-salicylic acid.

Degradation using absolute alcoholic potash has gone much better and yielded myristic acid and ω :4-dimethoxy-2-hydroxy-acetophenone in good yields. From these results it is clear that kanugin is a robinetin derivative and that it has a methylenedioxy group in the 3':4'-positions, the other three hydroxyl groups being protected by methyl groups (I).



The above constitution explains the peculiar results obtained by the treatment of kanugin with hydriodic acid and the re-methylation of nor-kanugin.

When purified kanugin is employed, the nor-kanugin obtained has slightly different properties from those already recorded. It is a yellow crystalline substance melting at about 325° with decomposition and its composition corresponds with the formula $C_{15}H_{10}O_7$. Its pentamethyl ether melts at $148-49^{\circ}$ and its penta-acetate at $223-25^{\circ}$. Its identity with robinetin has been established by comparison with a synthetic sample obtained by the method of Allan and Robinson, starting from ω -methoxy resacetophenone and the sodium salt and anhydride of *O*-trimethyl gallic acid, followed by demethylation.² Mixed melting point determinations of the flavonols and their derivatives have been made as also a comparison of the colour reactions of the two samples under identical conditions. The data are presented in the following table.

Property	Nor-kanugin	Robinetin
1. Melting point of the flavonol ..	320-25°	320-25°
2. Colour with FeCl ₃ ..	Dark olive brown	Dark olive brown
3. Precipitate with lead acetate ..	Red	Red
4. Colour changes at pH 11 ..	Quickly dissolves to a yellow solution, changes to emerald green in 10 seconds, then to greenish blue and pure blue, changes further to violet and finally pink, resembling alkaline phenolphthalein. This colour is stable for an hour and fades to brown through brown pink	
5. Melting point of the methyl ether ..	135-36° and 148-49°	135-36° and 148-49°
6. Melting point of the acetate ..	223-25°	223-25°

Some samples of the pentamethyl ether of nor-kanugin (natural) and of robinetin (synthetic) have been found to be melting at 148-49° and some others at 135-36°. But in the latter case, when the melt is allowed to solidify, the solid subsequently melts at 148°. A mixture of the low and high melting samples melts only at 148°. It may therefore be concluded that there are two forms of the solid methyl ether having two different melting points.

In the constitution of kanugin given above, two important features should be noted. Kanugin belongs to the small number of naturally occurring anthoxanthin derivatives in which all the hydroxyl groups are protected. But the more important point is the use of a methylene group for this protection, this being the first instance where this group has been found in the flavone series.

It may be interesting to compare the crystalline flavonol components present in the different parts of the *Pongamia* tree. The seeds³ contain karanjin which may be considered to be a derivative of 3:7-dihydroxy-flavone, *i.e.*, derived from resorcinol. The constitution of the other crystalline substance pongamol, also present in the seeds, is not yet clearly known. Kæmpferol is found in the flowers.⁴ It is also a flavonol but is derived from phloroglucinol. The constitution of pongamin which is found in very small amounts in the flowers is still unknown. The root bark⁵ contains kanugin which is again a flavonol derived from resorcinol but the side-phenyl nucleus is in a higher state of oxidation. Another noteworthy feature is that in the flowers the protection of the hydroxyl groups is the least whereas in the roots it is complete; an efficient mechanism should therefore be available in this part of the plant for this protection.

EXPERIMENTAL

Kanugin.

The sample of kanugin obtained from the root bark of *Pongamia glabra* was crystallised using excess of alcohol in which it is sparingly soluble. The

light colourless crystalline solid that was produced after two crystallisations melted between 196 and 200°. For further purification it was again crystallised twice using a mixture of alcohol and acetic acid. It then came out as colourless brittle rectangular plates and needles with a pearly lustre melting at 203–05°. Further crystallisation did not raise the melting point [Found: C, 63·8; H, 4·7; OCH₃, 25·8; C₁₉H₁₆O₇ requires C, 64·0; H, 4·5; OCH₃ (3), 26·1%]. It dissolved in concentrated sulphuric acid to give a bright yellow solution and the colour changes and fluorescence were exhibited as reported previously. Even an alcoholic solution gave a blue-violet fluorescence; with magnesium and hydrochloric acid a brilliant scarlet red colour was produced. When gently warmed with a solution of gallic acid in concentrated sulphuric acid, a deep emerald green colour was obtained in 10 seconds.

Alkaline Hydrolysis of Kanugin.

(1) *Using aqueous alcoholic potash.*—Kanugin (0·5 g.) was dissolved in aqueous alcoholic potash (alcohol 40 c.c., water 40 c.c. and potash 5 g.) and the solution boiled under reflux for 6 hours in an atmosphere of hydrogen. It was then cooled, acidified with hydrochloric acid and the alcohol distilled off under reduced pressure. The residue was then extracted repeatedly with ether. The ether solution was then shaken first with aqueous sodium bicarbonate in order to separate acid products (A) and subsequently with dilute sodium hydroxide to remove phenolic components (B). Neutral products would then be left in the ether solution (C). (B) and (C) were found to be insignificant in amount and were not further studied.

On acidifying the bicarbonate extract a solid separated out. This was washed with water and marked (D). By ether-extracting the solution the more soluble part was isolated (E). When (D) was crystallised from dilute alcohol it melted at 212–13° and had the appearance of long rectangular plates and prisms with a tendency to taper at the ends (Found: C, 55·3; H, 4·4; OCH₃, 16·3; C₉H₈O₅ requires C, 55·1; H, 4·1 and OCH₃, 15·8%). It formed a yellow solution in concentrated sulphuric acid; when a crystal of gallic acid was added and the solution gently warmed the colour changed to bright emerald green and finally to a stable pure blue. The mixed melting point with an authentic sample of myristic acid prepared from oil of nutmeg was not depressed.

When (E) was crystallised from boiling water it yielded a colourless crystalline product (flat needles) which had an indefinite melting point (130–45°), gave a purple colour with ferric chloride and had all the properties of a similar sample (4-methoxy-salicylic acid) already reported in Part I,

The substance (0.2 g.) was dissolved in glacial acetic acid (3 c.c.) and treated with a slight excess of a solution of bromine in acetic acid. The solution was rapidly decolourised and on keeping the mixture for a few hours at 0° a crystalline solid separated out. It was filtered and washed with a little water. Since it was very sparingly soluble in solvents it was purified by boiling with glacial acetic acid. It then melted at 258–59° with sintering a little earlier and had the appearance of small rectangular plates (Found: C, 38.8; H, 3.1; $C_8H_7O_4$ Br. requires C, 38.9; H, 2.8%). It was identical with a sample of 5-bromo-4-methoxy-salicylic acid prepared from a pure sample of 4-methoxy-salicylic acid.

The bromo acid was esterified by boiling with anhydrous methyl alcohol and a little concentrated sulphuric acid for 12 hours. On allowing to stand overnight crystals of the ester separated out. When recrystallised from methyl alcohol-acetic acid mixture it came out in the form of rhombohedral plates melting at 145–46°, agreeing with the description of the ester by Potter Rice⁶ who obtained it by a different method.

(2) *Using absolute alcoholic potash.*—Kanugin (0.75 g.) was treated with absolute alcoholic potash (30 c.c. of 8% solution) and the mixture refluxed for 6 hours under anhydrous conditions. The solid did not go into solution easily and only after 2½ hours solution was complete. After 6 hours, the solvent was completely removed, water (30 c.c.) added and the solution filtered through a plug of cotton-wool. When the filtrate was acidified, a crystalline solid separated in good yield. The solution along with the solid was extracted with ether twice and the ether solution shaken with aqueous sodium bicarbonate to separate the acidic component (A). The ether layer was finally washed with water and evaporated. The residue was a liquid which soon solidified. It was filtered, washed with a little water and crystallised twice from hot water when it came out as colourless thin rectangular plates melting at 65–67°. Mixed melting point with an authentic sample of ω : 4-dimethoxy-2-hydroxy-acetophenone was undepressed. It gave a reddish brown colour with ferric chloride and did not respond to the methylenedioxy group test. The 2:4-dinitrophenyl-hydrazone of the ketone prepared in the usual manner was crystallised from ethyl acetate, when it came out as bright red rectangular plates. It melted at 218–20° alone or when admixed with the 2:4-dinitro-phenylhydrazone of synthetic ω : 4-dimethoxy-2-hydroxy acetophenone.

On acidifying the bicarbonate extract (A) with hydrochloric acid a crystalline solid was obtained. It was filtered, washed with a little water and purified by crystallising twice from methyl alcohol from which it sepa-

rated as big rectangular prisms with a tendency to taper at the ends and melting at 212–14°. This was identical with myristic acid obtained in the other method of hydrolysis

Demethylation of Kanugin: Nor-Kanugin (Robinetin).

A solution of kanugin (0.5 g.) in phenol (3 c.c.) was treated with hydriodic acid (10 c.c. of d. 1.7). The mixture was heated at 150–60° for 1 hour. It was then cooled, diluted with water (50 c.c.) and free iodine decomposed by means of sodium sulphite. The yellow solid that separated out was filtered, washed repeatedly with hot water and purified by crystallising it from aqueous alcohol when it came out as yellow rectangular plates, melting at 320–25° with decomposition (Found in the air-dried sample: C, 55.9; H, 4.1; $C_{15}H_{10}O_7$, H_2O requires C, 56.2; H, 3.7%). The compound was sparingly soluble in hot water and moderately in alcohol and acetic acid. Its alcoholic solution exhibited a brilliant green fluorescence. It gave a dark olive green colour with ferric chloride and a red precipitate with lead acetate in alcoholic solution. In concentrated sulphuric acid it dissolved to form a yellow solution without fluorescence.

Nor-Kanugin Acetate

The acetate of nor-kanugin was prepared by boiling it (0.1 g.) with acetic anhydride (3 c.c.) and a drop of pyridine for an hour and a half. The anhydride was then removed under reduced pressure and the white solid left behind was purified by crystallisation from absolute alcohol. It came out in the form of colourless narrow rectangular prisms, melting at 223–25°. The mixed melting point with a sample of the acetate of robinetin was not depressed (223–25°).

Nor-Kanugin Methyl Ether

A solution of nor-kanugin (0.1 g.) in anhydrous acetone (25 c.c.) was treated with dimethyl sulphate (0.3 c.c.) and anhydrous potassium carbonate (2 g.). After refluxing for 8 hours, the potassium salts were filtered off and the residue washed with a little acetone. When the solvent was distilled off, the methyl ether was obtained as a white crystalline solid. It was purified by crystallisation from alcohol when it appeared as narrow rectangular plates melting at 148–49°; the mixed melting point with *robinetin* methyl ether (synthetic) was undepressed (Found in the air-dried sample: C, 61.9; H, 6.0; OCH_3 , 38.9 and loss on drying *in vacuo* 5.0. $C_{20}H_{20}O_7$, H_2O requires C, 61.5; H, 5.6; OCH_3 39.7 and H_2O loss 4.6. Found in samples dried at 110° *in vacuo*: C, 64.9, H, 5.5; $C_{20}H_{20}O_7$ requires C, 64.5; H, 5.4%).

SUMMARY

Kanugin $C_{19}H_{16}O_7$ yields myristic acid and *p*-methoxy salicylic acid when decomposed with aqueous alcoholic potash and myristic acid ω :4-dimethoxy-2-hydroxy-acetophenone when decomposed with absolute alcoholic potash. Nor-kanugin has been identified as robinetin by a comparison of the flavonols and their derivatives. It is, therefore, concluded that kanugin is 3:7:5'-trimethoxy-3':4'-methylenedioxy-flavone. The crystalline components of the seeds, flowers and root bark of *Pongamia glabra* are compared.

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