

CONSTITUTION OF KANUGIN—PART I

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THE isolation of kanugin, a new crystalline compound from the roots of *Pongamia glabra* has been described recently.¹ It melts at 197-98° and has the molecular formula $C_{19}H_{18}O_7$. It is a neutral compound and does not contain any phenolic hydroxyl groups. It however has three methoxyl groups and seems to be a completely methylated compound. Since it gives a deep red colour by reduction with magnesium and hydrochloric acid it may belong to the group of methoxyflavones. It has now been found to be feebly toxic to fish. The results of some experiments carried out with a view to understand the constitution in greater detail are described in the present communication.

Demethylation of kanugin by boiling with hydriodic acid in the absence of air gave a sparingly soluble yellow crystalline compound which did not melt below 310° and which had the probable formula $C_{16}H_{12}O_7$. Its ready solubility in cold dilute sodium hydroxide and the formation of an olive brown colour with ferric chloride, a red precipitate with neutral lead acetate and a scarlet colour on reduction with magnesium and hydrochloric acid showed it to be a compound of the flavonol group. The rapid colour changes which it exhibited in alkaline buffer solutions of higher pH lent support to this conclusion. But the partial recovery of the compound after aerial oxidation in alkaline solution (50% potash) did not quite fit in with the above view. Probably there is something in the nature of the molecule of nor-kanugin which is compatible with all the above observations and which is not at present clear.

The acetate of nor-kanugin prepared by boiling with acetic anhydride and sodium acetate melted at 198-99° which was almost the same as the melting point of kanugin itself. The interesting observation relating to this compound was that when methylated using dimethyl sulphate and alkali in acetone solution it gave rise to a product which was not identical with kanugin. The new compound melted at 153° and the crystal forms were not identical. It also gave a red colour on reduction with magnesium and hydrochloric acid showing that it belonged to the same general class of compounds (flavones). The formation of a new methyl ether from the acetate could be explained only on the assumption that some

complex changes had taken place during either the demethylation of kanugin or the acetylation or the subsequent remethylation of nor-kanugin. In all probability the treatment of kanugin with hydriodic acid resulted not only in demethylation of the methoxyl groups but in the liberation of certain new phenolic groups which were not present in kanugin.

By the oxidation of kanugin with neutral potassium permanganate in acetone solution two degradation products were obtained. One was identified as *p*-methoxysalicylic acid by a direct comparison with an authentic specimen obtained by the methylation of β -resorcylic acid with dimethyl sulphate and alkali. The other product which was neutral, being insoluble in cold sodium hydroxide, melted at $135-40^\circ$ and had the formula $C_7H_8O_2$ or $C_{11}H_{14}O_3$. It also contained methoxyl groups. The available quantity of this compound was not sufficient for further characterisation.

The hydrolysis of kanugin with aqueous alcoholic potassium hydroxide gave rise to a considerable yield of an acid which has resisted all attempts at purification so far. It was definitely crystalline in appearance and melted indefinitely in the neighbourhood of 130° and gave a purple colour with ferric chloride. These properties together with the percentage composition (the analysis corresponds to the formula $C_8H_8O_4$) indicated that it was probably an impure sample of *p*-methoxysalicylic acid which, as mentioned above, had been obtained by the oxidation of kanugin. Possibly it was closely associated with another compound of similar composition and similar properties which could not be eliminated by the ordinary methods of crystallisation. The observation that a mixture with *p*-methoxysalicylic acid melted indefinitely between the melting points of the two components is in agreement with the above view.

The following points emerge from the above observations. Kanugin has a general resemblance to the flavones, a group of compounds which are found widely distributed in nature. It has a resorcinol unit in which one of the hydroxyl groups is present in the form of the methyl ether. Treatment with hydriodic acid brings about some complex change possibly involving the breakdown of an oxygen bridge.

Although these results by themselves do not give any definite idea of the constitution of kanugin they are presented now, since as a result of conditions arising out of the war progress is slow and it may take considerable time to get more definite data.

Experimental

Demethylation of kanugin to nor-kanugin.—The compound (0.2 g.) was dissolved in phenol (3 c.c.) and treated with hydriodic acid (d. 1.7, 5 c.c.).

The mixture was heated for two hours in an oil-bath at 150° with a gentle current of carbon dioxide passing through the reaction vessel. It was then diluted with water (25 c.c.) and decolourised with sulphur dioxide. The yellow solid was filtered, washed with water and crystallised from acetic acid containing a few drops of pyridine. The compound thus obtained was a yellow crystalline powder (clusters of needles and narrow rectangular plates) which turned dark at 290° but did not melt below 310° (Found : C, 60.9 ; H, 3.6 ; $C_{16}H_{12}O_7$ requires C, 60.8 H, 3.8%.)

Nor-kanugin was sparingly soluble in alcohol and acetic acid and easily soluble in pyridine. It dissolved readily in cold dilute sodium hydroxide giving a deep green solution. An alcoholic solution of the substance gave a dark olive brown colour with ferric chloride and a red precipitate with neutral lead acetate. Reduction with magnesium and hydrochloric acid in alcoholic solution gave a scarlet colour. On the other hand using sodium amalgam as the reducing agent the following changes were observed : the solution became red after reduction had taken place; on acidifying with concentrated hydrochloric acid the solution became colourless while with a large excess of concentrated sulphuric acid a brown colour was seen. In Bargellini's test greenish blue flocks were first formed; on shaking the test tube the solution was filled with a deep green precipitate which rapidly gave place to a yellowish brown clear solution. The following changes were noticed when small quantities were shaken with buffer solutions of different pH values :

- p_H 8.0 Partly dissolved to give a pale yellow solution. Turbid even after 3 minutes. Unchanged after half an hour. Turbid and unchanged even after 48 hours.
- p_H 9.2 Dissolved easily on shaking to form a golden yellow solution. Orange tinge after half an hour. Brighter orange after 1 hour. Unaffected even after 48 hours.
- p_H 10.4 Yellow solution rapidly changing to green in half a minute. Greenish yellow in one minute. Yellowish brown after 4 minutes. Orange red after half an hour. Brown after 48 hours.
- p_H 11.6 Deep yellow solution. Emerald green in 10 seconds. Brownish red in 2 minutes. Red in 4 minutes. Deep scarlet after 10 minutes. Unchanged after 1 hour. Brown after 48 hours (deeper than with pH 10.4).
- p_H 12.8 Deep yellow solution. Emerald green in 5 seconds. Very pale yellowish brown in $\frac{1}{2}$ min. Brown red in 1 min. Red in 2 min. Deep scarlet after 5 min. Unchanged after 1 hour.

Brown after 48 hours (same as with pH 11.6). The changes in this case were the same as with pH 11.6 but much quicker.

Nor-kanugin Acetate:

The acetate was prepared by boiling with sodium acetate and acetic anhydride for 4 hours and pouring the mixture into excess of water. The solid was filtered, washed and crystallised from alcohol-acetic acid mixture. Stout needles were obtained but as the mother-liquor was highly coloured another crystallisation was effected with the above solvent mixture; this time the mother liquor was practically colourless. The substance crystallised as colourless stout needles and melted at 198-99°, with sintering at 193°. A mixed melting point with kanugin was considerably depressed. [Found: C, 60.4; H, 4.0; $C_{16}H_{12}O_7$ (C_2H_2O)₃ requires C, 59.7; H, 4.1; $C_{16}H_{12}O_7$ (C_2H_2O)₅ requires C, 59.3; H, 4.2%.]

Methylation of nor-kanugin acetate:

The acetate (150 mg.) was dissolved in acetone (20 c.c.) and treated with methyl sulphate (2 c.c.) and alkali (2 c.c. of 20% sodium hydroxide solution). After shaking for half an hour further quantities of the reagents were added (1 c.c. of each at the end of every half hour). When 6 c.c. of methyl sulphate had been added in all, 4 more c.c. of 20% sodium hydroxide were added and the mixture was left overnight. After refluxing for half an hour the solvent was evaporated. On cooling, a pale brown solid separated which was filtered, washed and crystallised easily from alcohol. Narrow rectangular plates and tablets were thus obtained which melted sharp at 153°.

The new methyl ether was a colourless shining solid appearing as long needles to the naked eye. It was soluble sparingly in cold alcohol and easily in hot alcohol. It dissolved easily in cold concentrated sulphuric and hydrochloric acids to give yellow solutions having a feeble green tinge. It was insoluble in dilute aqueous sodium hydroxide even after boiling for 3 minutes and gave no colour reaction with alcoholic ferric chloride. Reduction in alcoholic solution with magnesium and hydrochloric acid gave a red colour.

Aerial oxidation of nor-kanugin:

The compound (50 mg.) was shaken with 50% aqueous potash (2 c.c.) in the presence of air. The solution which was intense red and opaque in the beginning became less opaque gradually, though there was no great change in the colour. After leaving in an open test tube with occasional shaking for 48 hours it was acidified, ether-extracted and the slightly sticky residue obtained on evaporation of the solvent examined. It was yellow in colour, gave the same reduction test with magnesium and hydrochloric acid as the

original compound and the same reactions with ferric chloride and lead acetate. It showed no tendency to melt when heated up to 310° , and thus appeared to be an impure sample of the original compound.

Oxidation of kanugin with neutral permanganate :

The compound (0.5 g.) was dissolved in acetone (75 c.c.) and an aqueous solution of potassium permanganate (2 g. in 50 c.c. of water) was added during 4 hours with continuous shaking. The solution remained purple in the initial stages but subsequently became turbid and brown owing to precipitation of manganese oxide. Considerable warmth was also developed during the later stages. After leaving overnight the mixture was saturated with sulphur dioxide. A dark coloured oil separated at the bottom, leaving a clear, colourless liquid above. The solvent (acetone) was evaporated from a water-bath (about 20 minutes). At first the coloured oil slowly dissolved but subsequently a white solid separated. After heating for another 15 minutes the mixture was cooled and repeatedly ether extracted, filtering off any insoluble matter. On evaporation of the combined ether extract a yellow mass of crystals was obtained. It was heated with aqueous alcohol and a small quantity of a sparingly soluble substance was discarded. The clear alcoholic filtrate deposited on cooling a very small quantity of a solid which was removed by filtration. Concentration of the residual clear alcoholic solution gave rise to a turbid liquid with a few oily drops. On stirring with a little aqueous sodium bicarbonate the oil dissolved partly leaving a solid (A) behind.

Solid A : Neutral compound.—This was filtered, washed and crystallised from aqueous alcohol. Narrow rectangular plates and tablets melting at $135-40^{\circ}$ were obtained. The compound was sparingly soluble in hot water and insoluble in sodium bicarbonate and sodium hydroxide. It did not give a ferric chloride colour, but gave tests for methoxyl groups. (Found : C, 68.0; H, 7.1; $C_7H_3O_2$ requires C, 67.8; H, 6.5%; $C_{11}H_{14}O_3$ requires C, 68.1; H, 7.2%.)

Acidic component : p-methoxysalicylic acid.—On acidifying the sodium bicarbonate solution (from A above) with hydrochloric acid a colourless solid was obtained. It was crystallised from hot water discarding a small amount of resin and non-crystalline solid which came down on cooling to 80° . The fine shining needles that subsequently separated melted at 155° , appeared as rectangular rods with tapering ends under the microscope and gave a deep purple colour with alcoholic ferric chloride. A comparison with an authentic specimen of *p*-methoxysalicylic acid prepared by methylating β -resorcylic acid with dimethyl sulphate and alkali showed that the two were identical.

Alkali fission of kanugin.—The compound (0.5 g.) was treated with alcohol (25 c.c.), water (25 c.c.) and potassium hydroxide (2.5 g.) and the mixture was heated under reflux in an atmosphere of hydrogen. The solid dissolved slowly in about half an hour giving a clear reddish yellow solution. After boiling for 4 hours and cooling in the atmosphere of hydrogen the solution was acidified with hydrochloric acid, the alcohol distilled off and the residue ether extracted. The ether extract was shaken successively with aqueous sodium bicarbonate (a) and dilute sodium hydroxide (b) and then evaporated (c). The sodium bicarbonate solution (a) when acidified gave a copious precipitate of a colourless crystalline solid which was filtered and recrystallised from hot water. Though it was quite crystalline to the naked eye and under the microscope (fine needles) the melting point was not quite satisfactory. It sintered at 122° and melted at 128-30°. Attempts to recrystallise it from various solvents so as to get a sharp melting compound have not so far been completely successful though the melting point could be raised to 145°. It was a carboxylic acid, dissolving easily in cold aqueous sodium bicarbonate to give a yellow solution and it gave a purple colour with ferric chloride in alcoholic solution. (Found: C, 57.7; H, 4.9, OCH₃, 17.5; C₈H₈O₄ requires C, 57.2; H, 4.8, OCH₃ (1), 18.5%.)

(b) The sodium hydroxide solution when acidified did not give rise to any solid or liquid. Hence it was extracted with ether and the solvent evaporated. The residue was insignificant and it could not be examined.

(c) When the residual ether solution was evaporated, an oil separated in small amount but it did not solidify even after two days. Treatment with phenylhydrazine and acetic acid did not give rise to any definite product.

Summary

By the oxidation of kanugin with potassium permanganate in acetone solution 4-o-methylresorcylic acid (I) and a neutral compound (II) containing methoxyl groups and having the formula C₇H₈O₂ or C₁₁H₁₄O₃ have been obtained. Hydrolysis with alcoholic alkali yielded an acid which seemed to be impure (I). The presence of a resorcinol unit in the molecule seemed to be thus established. Demethylation of kanugin gave rise to nor-kanugin. It had the properties of a flavonol (C₁₆H₁₂O₇) and gave bright colours in alkaline buffer solutions. When its crystalline acetate was methylated a new methyl ether which was different from kanugin was produced. It was therefore concluded that by the action of hydriodic acid, besides demethylation some other changes were brought about.

REFERENCE

1. Rangaswami and Seshadri .. *Proc. Ind. Acad. Sci., A*, 1942, 16, 319.