

CHEMICAL EXAMINATION OF JACK TREE LATEX AND JACK FRUIT GUM

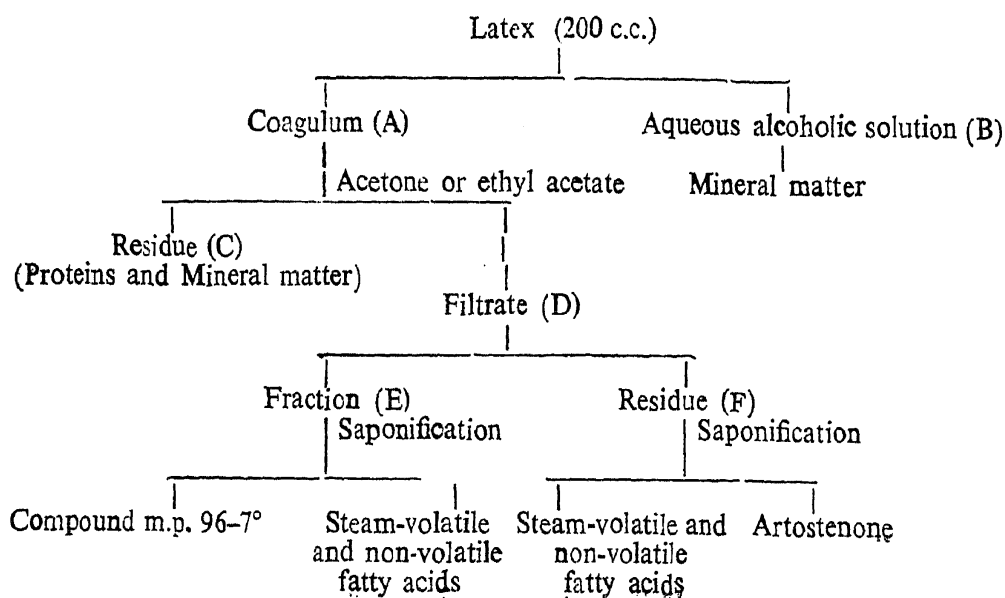
Part I. The Origin of Artostenone

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JACK TREE (*Artocarpus integrifolia*) readily yields a milky latex when the green stem is cut or when a twig or leaf is broken off. During the war there was great interest in the study of plant latices and we had occasion to examine a small quantity of the Jack tree latex. It was coagulated by adding just enough of alcohol (equal volume) and the resulting coagulum (A) and aqueous alcoholic mother liquor (B) examined (see latex of *Calotropis gigantea*¹). The latter (B) contained no special components. From the former (A) by treatment with acetone or ethyl acetate an insoluble portion (C) was separated and found to contain mineral salts and proteins. The acetone or ethyl acetate soluble portion (D) consisted mainly, of waxy matter. A sparingly soluble fraction (E) of this melted at 84°, gave no tests for sterols or resinols and on saponification yielded higher fatty acids and a substance melting at 96–7° and having the composition $C_{30}H_{58}O_2$. But the remaining fraction (F) gave strong reaction for sterols and on saponification yielded fatty acids, volatile as well as non-volatile. The unsaponifiable portion on repeated crystallisation provided a crystalline, sharp melting compound which was identified as artostenone² from a



detailed study of its properties and reactions. In the course of the preparation of an authentic sample of artostenone for purposes of comparison we also examined the gum obtained from the fruit and found that it closely resembled the tree latex in composition.

Thus artostenone is the main isolable crystalline component of the latex and fruit gum. Since it has been established to be a ketone with no hydroxyl group it should be occurring free and preliminary saponification of the waxy matter should not be absolutely necessary. But all attempts to isolate it from the waxy matter by simple crystallisation from various solvents were unsuccessful. Chromatography was next employed. Though free artostenone gets readily adsorbed on alumina, the waxy matter of the latex and gum was only very weakly adsorbed and no separation of artostenone could be effected. It therefore appeared that this ketone is not occurring free as such, but is present in a modified form capable of forming a wax ester.

In recent years enol esters of steroid ketones have been prepared by a number of workers³ and it appeared to be possible that an enolic isomer of artostenone may be present in the waxy matter as an ester. The methods adopted by previous workers for preparing enol benzoates were examined but were found to produce no change in artostenone. But α -artostenone² which is obtained by the action of alcoholic sulphuric acid on artostenone readily forms an enol-benzoate. This enol-ester as well as α -artostenone undergo conversion into artostenone under the alkaline conditions prevalent during saponification. It therefore seems to be clear that artostenone does not occur in the plant, but α -artostenone in the form of wax esters is present and hence the need for the preliminary saponification during which the transformation into artostenone takes place.

In the course of this work we could isolate by repeated crystallisation a fairly sharp melting wax fraction which on saponification yields artostenone. But as in the case of other waxes this may be only a mixture of esters and not a simple substance. Among the acids volatile and non-volatile components have been found. The former are markedly offensive smelling indicating the existence of isovaleric acid. Definite tests for the presence of acetic acid are also given by this portion.

EXPERIMENTAL

Tree latex

The latex coming out of incisions on the trunk and branches of the tree was collected in bottles containing chloroform to prevent any possible decomposition. 200 c.c. of it was treated with an equal volume of alcohol

and the mixture stirred well and allowed to stand when a sticky solid settled down. The coagulum (A) was separated from the aqueous alcoholic solution (B) by filtration.

The coagulum (A) was dried in the sun (50 g.) and digested repeatedly with boiling ethyl acetate or acetone. As part of it was insoluble, the hot solution was filtered and the residue (C) on the filter was examined separately. The filtrate (D) was allowed to cool slowly when a colourless solid (E) separated out. It was filtered off and the filtrate was concentrated by removing the solvent under reduced pressure. The residue (F) was obtained as a sticky, brown semi-solid.

Fraction F

Attempts to crystallise this fraction from benzene-alcohol or acetone-alcohol mixture did not yield a crystalline product. It (25 g.) was therefore dissolved in benzene (150 c.c.), an equal volume of 8% alcoholic potash added and the mixture boiled under reflux for 20 hours. The solvents were distilled off on a water-bath and the concentrate was treated with water when a brown semi-solid separated out slowly. This unsaponifiable matter (10.0 g.) was extracted with ether and the ether solution was dried over anhydrous sodium sulphate. When the solvent was removed by distillation a reddish-brown semi-solid was left behind. It was dried in a vacuum desiccator over concentrated sulphuric acid and purified by repeated crystallisation from alcohol-acetone mixture, benzene-alcohol mixture and alcohol. It was thus obtained in the form of colourless large rectangular plates melting at 109°–110°; yield 1.5 g. (Found C, 85.0; H, 11.8; $C_{30}H_{50}O$ requires C, 84.5; H, 11.7%.)

The substance had all the properties and gave the colour reactions of artostenone (Nath²) and also formed an oxime melting at 175°. The mixed melting points of the ketone and of its oxime with authentic samples obtained from the fruit gum were not depressed.

The soap from the above saponification was decomposed with sulphuric acid. The fatty acids that were liberated were resolved into the steam-volatile and non-volatile fractions. The former had a markedly unpleasant odour resembling that of isovaleric acid and it also gave the lanthanum nitrate test for acetic acid. The non-volatile acids melted at about 65°.

Fraction E

On recrystallisation from ethyl acetate this was obtained as a hard nodular solid melting at 84°. It did not respond to the usual sterol or triterpene Colour reactions. Further purification through crystallisation did not bring

about any change either in its appearance or in its melting point. Hence it was saponified in benzene solution with alcoholic potash. The unsaponifiable matter, on repeated crystallisation from acetone and alcohol, was obtained as a well-defined crystalline (colourless tiny plates) compound melting at 96–7°. Further purification through crystallisation did not change either the melting point or the crystal structure. It did not respond to the characteristic colour reactions of sterols or resinols. However, it dissolved in concentrated sulphuric acid to produce a yellow solution exhibiting green fluorescence. The yield of the compound was small (0.2 g.) when compared with that of artostenone. (Found: C, 80.5; H, 13.4; $C_{30}H_{58}O_2$ requires C, 80.0; H, 13.0%.)

The soap from the above saponification was decomposed with sulphuric acid and the fatty acids were divided into steam-volatile and non-volatile fractions. The former closely resembled the fraction obtained from (F). The non-volatile acid mixture melted round about 80°.

Fraction C

The acetone or ethyl acetate insoluble portion was obtained in the form of a fine powder after drying for 2 or 3 days at room temperature. It was completely soluble in water and yielded about 8% of ash which contained phosphates and sulphates as acid radicals and calcium and iron as metallic radicals.

The organic part which was also soluble in water gave tests for proteins and the reactions showed that they belonged to the class of albumins and globulins.

Aqueous alcoholic solution (B)

When it was diluted with water no solid separated out. Further extraction with chloroform and ether did not yield any material. On acidifying it with hydrochloric acid there was no precipitate. The solution was found to contain mainly phosphates and sulphates as acid radicals and calcium and iron as metallic radicals.

Fruit gum

The gum was obtained from Malabar and was collected from the ripe fruit. It was dried first in the sun and then at 100° before use. It (250 g.) was fractionated and examined in detail exactly in the same manner as in the case of the coagulum (A) from the tree latex.

Extraction with ethyl acetate or acetone left behind fraction (C) consisting of mineral matter and proteins. These were similar to those obtained

in the corresponding fraction of the tree latex. The solution (D) on moderate concentration yielded fraction (E). This fraction, on saponification, yielded the compound melting at $96-7^{\circ}$ and having the formula $C_{30}H_{58}O_2$. The yield of the compound in this case also was poor (0.5 g.). On removing the solvent completely under reduced pressure from the solution (D), fraction (F) (100 g.) was obtained. This was saponified. From the unsaponifiable matter (50 g.), 5 g. of pure artostenone could be obtained by repeated crystallisation. The steam-volatile and non-volatile acids from the soap solution were similar to those obtained from the corresponding fraction in the case of the tree latex.

Chromatography experiments

Artostenone (0.5 g.) was dissolved in benzene (50 c.c.) and was passed down a column (10 cm. by 1 cm.) of alumina under gentle suction. All the substance was adsorbed. Repeated elution with the same solvent brought out all the compound. It melted at $109-110^{\circ}$ and no difference was found between different fractions.

The waxy solid fraction (F) (2 g.) was dissolved in benzene (100 c.c.) and subjected to chromatography as before. There was very poor adsorption and the portion adsorbed was also waxy like the unadsorbed matter though less pure. When the major unadsorbed portion was crystallised from a mixture of acetone and alcohol it was obtained as a colourless solid melting at $80-82^{\circ}$. This substance on saponification with alcoholic potash gave artostenone melting at $109-110^{\circ}$.

α -Artostenone

This was prepared according to the procedure of Nath², by heating artostenone with alcohol and sulphuric acid for 4 hours. When crystallised twice from alcohol it was obtained in the form of colourless glistening plates melting at $99-100^{\circ}$. The mixed melting point with artostenone was depressed ($85-90^{\circ}$).

Reconversion of α -artostenone to artostenone

α -Artostenone (0.1 g.) was dissolved in 4% absolute alcoholic potash and the solution refluxed for four hours. The alcohol was removed by distillation and the residue treated with water. A colourless product separated out and it was filtered off. When crystallised twice from alcohol, it was obtained as shining rectangular plates melting at $109-110^{\circ}$; the mixed melting point with artostenone was undepressed.

α -Artostenone-benzoate

α -Artostenone (1.0 g.) was dissolved in pyridine (10 c.c.) and benzoyl chloride (10 c.c.) added. The mixture was refluxed for 20 minutes, cooled and treated with water. It was set aside until there was no smell of benzoyl chloride. The aqueous solution was then decanted off and the brown sticky solid left behind was stirred up with a little methyl alcohol. It was filtered and crystallised from a mixture of benzene and alcohol (1:3). The benzoate was obtained as clusters of fine colourless needles melting at 157–59° (Found: C, 83.5; H, 10.0; $C_{37}H_{54}O_2$ requires C, 83.8; H, 10.2%).

Hydrolysis of α -Artostenone-benzoate

α -Artostenone benzoate (0.5 g.) was dissolved in 5% alcoholic potash (10 c.c.) and the mixture was refluxed for 3 hours. The alcohol was removed by distillation and the residue taken up in water. The solid that separated out was filtered and washed free from alkali. On crystallisation from alcohol it melted at 109–110° and the mixed melting point with artostenone was undepressed.

SUMMARY

Jack tree latex has the same composition as the fruit gum. Besides proteins and mineral substances, the two contain a high percentage of waxy matter. The less soluble portion (small amount) of the wax yields on saponification a non-steroid substance melting at 96–7° and having the probable composition of $C_{30}H_{58}O_2$. The more soluble major fraction yields artostenone on saponification. Experiments indicate definitely that this ketone is not occurring free and that it is formed as a result of the saponification. Though artostenone does not have the capacity of forming enol-esters, α -artostenone yields an enol-benzoate readily. Further α -artostenone and its enol-ester undergo conversion into artostenone under the conditions of saponification. Hence the conclusion is drawn that in the plant it is α -artostenone that occurs in the form of enol-wax-esters.

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