

CHEMICAL INVESTIGATION OF INDIAN FRUITS

Part I. Bitter Principles of Pamparapanas (Indian Shaddock)

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Received May 18, 1940

THE bitter taste of the fruits of the citrus group has been attributed to the existence of certain crystalline compounds the most important of which is naringin. Their study is of great interest in connection with the fruit industry. Though some element of bitterness is agreeable too much of it is a serious disadvantage. Two important points to be noted in this connection are (1) the bitter principles are found to reside mostly in the peels, rags (placenta and vascular bundles) and the seeds and (2) they are capable of conversion into non-bitter derivatives. The second phenomenon takes place particularly when the fruit ripens or when it is stored. There is therefore large scope for producing fruit juices without much bitterness by suitably adjusting the conditions. Further, the by-products, peels and seeds have found use in medicine mainly because of the existence of bitter principles in them.

Pamparapanas (Indian Shaddock) is a very large sized fruit commonly available in the coastal tracts. The peels are thick, with definite flavedo and albedo and the cellulose coatings enclosing the different sections can be easily removed by hand. The seeds are large and have a rough surface. The peels and rags are found to contain naringin as the bitter principle and in this respect this fruit resembles the grape fruit. In appearance, the nature of the different parts and other characteristics, however, the two fruits differ markedly. Regarding the peels of pamparapanas the method that is usually advocated for the preparation of naringin from the peels of the grape fruits is not quite suitable.¹ A preliminary extraction of the dry sample with ether considerably improves the efficiency of the subsequent extraction with aqueous alcohol. This is due to the removal of the oily and resinous matter. The above operation is not necessary in the case of the rags since they contain very little of oil in them. The fresh wet rags contain a high percentage of naringin (1%) and hence during manufacture of the juice great care has to be taken to avoid crushing these rags and extracting them.

The seeds from which the oil had been removed by extraction with light petroleum were employed for studying the bitter principles. Further extraction with alcohol removed all the bitterness of the seeds. The bitter components could be divided into two parts: (1) water-soluble and (2) water-insoluble. The first portion was identified as naringin and the second as a mixture of limonin and isolimonin. It may be remarked here that the presence of the last two in orange kernels was noted by Koller and Czerney² and that Feist and Overberg³ recorded the presence of limonin in lemon pips. The occurrence of naringin in citrus seeds does not seem to have been observed till now. The point of interest is that naringin is intensely bitter and because of its solubility in water its taste is readily felt. On the other hand limonin and isolimonin are sparingly soluble in water and hence the solids when placed on the tongue do not give rise to the bitter taste. However when an alcoholic solution is diluted with a large volume of water, the dilute solution tastes markedly bitter. It is possible therefore that the marked bitterness of the seeds is mainly due to the naringin though the others may also add to it.

Naringin isolated from the different parts of the pamparapanas fruit was identified by a detailed study of its composition, properties and reactions and by comparison with an authentic sample of grape fruit naringin. The investigation definitely establishing the final constitution of naringin as a glycoside having the disaccharose group in the seventh position of naringenin has already been reported.⁵ Though limonin has been known for a long time as the bitter principle of lemon seeds the first detailed investigation was published by Koller and Czerney² in 1936. They obtained it from orange kernels and gave the molecular formula as $C_{23}H_{26}O_7$, the melting point as 280° and the specific rotation as -142° . Immediately after, Feist and Overburg³ gave a brief account of the properties of citrolimonin obtained from lemon seeds. This seems to be the same as limonin. The melting point however was given higher as 302° , the rotation as -135° and the molecular formula as $C_{26}H_{30}O_8$. Koller and Czerney also isolated a small quantity of a second bitter principle called isolimonin to which they gave the molecular formula $C_{23}H_{28}O_7$. More recently Higby⁴ has reported that isolimonin occurs as the main bitter principle of the peels, rags and seeds of Navel oranges and limonin in Valencia oranges, that the two are isomeric and that the former yields on hydrolysis with alkali limonin as one of the products.

The seeds of the shaddock probably owe their main part of the bitterness to the presence of naringin. However a mixture of limonin and isolimonin occurs to the extent of about 0.6% of the dry weight of the seeds and of

this isolimonin is only in very small quantities, about 0.03%. The separation of the two water-insoluble bitter compounds was effected by utilising the marked differences in solubility in alcohol and benzene. Our results relating to the composition and properties of limonin agree with those of Higby. The correct melting point of limonin seems to be 292° and the molecular formula $C_{26}H_{30}O_8$. The specific rotation recorded by Koller *et al.* and Feist *et al.*, is too high and differs considerably from the values obtained by Higby. We were first under the impression that this was due to the different solvents that they employed. On examining this point using acetone, chloroform and acetylene tetrachloride the correct rotation was found to be only about 110°, the high error of the early investigators being obviously due to the small quantities of substances that they employed and the volatility of the solvent.

The resemblance between limonin and isolimonin is rather close. They give the same reactions with acids and alkalies and have more or less the same rotation. Both seem to possess 2 lactone groups. Hence they should possess very closely related molecular constitutions. This is further supported by the fact that Higby has been able to prepare limonin from isolimonin. Besides melting point and solubility, other differences do not seem to be very definite. A number of attempts were made to decide if coumarin rings existed in the molecule of limonin. Experiments carried out under various conditions using methyl alcohol, potash and dimethyl sulphate did not yield any product of methylation. Hence the presence of α -pyrone rings is doubtful though not impossible.

Experimental

The fruits were obtained from Rajahmundry during the months of September and October. As soon as they arrived in the laboratory they were peeled out, the sections opened and the rags and seeds separated from the juicy portions as much as possible. The peels and the rags were preserved separately in wide-mouthed bottles with a little formalin. The seeds were dried and stored.

Analysis of the Peels.—The entire peels including the albedo and flavedo were employed. The fresh samples were cut into bits, crushed in a mortar and analysed adopting the methods of the Association of Official Agricultural Chemists for feeding stuffs. The results are presented in the form of a table along with those obtained for grape fruit peel by Poore.¹

	Fresh peels of Pamparapanas per cent.	Grape fruit peels (fresh) per cent.
Total solids	24·12	16·71
Mineral matter	2·08	0·74
Acids as citric acid	1·67	0·74
Crude fat (ether extract)	0·27	0·28
Proteins (N × 6·24)	1·51	1·13
Sugars as invert sugar	4·54	6·35
Starch as invert sugar	6·55	..
Pentosans	2·82	0·83
Crude fibre	3·86	1·71
Naringin	0·13	0·4
P ₂ O ₅	0·20	..
K ₂ O	0·43	..

The peels of the shaddock contain more solid matter and a higher proportion of carbohydrates, proteins and mineral salts and hence they are better as cattle fodder when large quantities are available as by-products in industry than grape fruit peels. Further they contain less of the bitter principle, naringin and hence are less objectionable.

Isolation of Naringin from the Peels.—The method of Poore¹ employing the fresh peels of the grape fruits and using methylated spirits for extraction was not found to be suitable in the present case. There was considerable difficulty in the separation of naringin from the extract due to the presence of oily and resinous matter. This was overcome by employing the dried peels and subjecting them to a preliminary extraction with ether.

Sun dried peels (800 g.) were ground to powder in a laboratory mill and kept soaked in ether for 12 hours. The ether extract was decanted and more ether added to the residue. The process was repeated twice again in order to remove as completely as possible ether-soluble matter. The ether extract was distilled to recover the solvent and the residue was examined. It did

not taste bitter and hence was discarded. The residual peels were then boiled under reflux with methylated spirits (about 1 litre) for two hours and the extract filtered. The solid residue was twice again extracted with alcohol in a similar manner and the combined extracts were treated with a slight excess of a saturated solution of neutral lead acetate. The precipitate that was formed was filtered, the filtrate heated nearly to boiling and a current of hydrogen sulphide passed in order to precipitate the excess of lead. After removing lead sulphide the clear solution was concentrated under reduced pressure to syrupy liquid. It was quickly filtered while hot and allowed to crystallise in an ice chest. After standing for about a week the crystals were collected and recrystallised from a small quantity of water. The substance was exceedingly bitter, appeared as colourless rectangular plates and melted at 83°. The yield of the pure substance was 0·13% of the fresh peels and 0·54% of the dry sample.

Naringin from the Rags.—The rags were far more bitter than the peels and the extraction of the bitter principle was easier for the reason that there was very little of oily matter. Preliminary drying and extraction with ether were therefore unnecessary. The fresh and wet rags were crushed in a mortar, extracted with boiling alcohol and the bitter principle isolated as in the case of the peels. The yield of the crystalline material was rather high (1%) and the substance was identical with the one obtained above.

The crystalline bitter substance was soluble to some extent in cold water, readily in boiling water, acetone and alcohol and insoluble in ether, light petroleum and benzene. It gave a yellow colour with aqueous sodium hydroxide, red colour with ferric chloride or with a reducing mixture of magnesium and hydrochloric acid. In the air-dry condition it melted at 83° but after drying at 110° the melting point was 172°. In alcoholic solution $[\alpha]_D$ was 89·5°. On hydrolysis with dilute sulphuric acid it gave a non-bitter aglucone melting at 244°. The sugars that were produced as the other products of hydrolysis were recognised as glucose and rhamnose from their osazones. From all these properties the substance was identified as naringin and this was confirmed by comparison with an authentic sample of grape fruit naringin.

The Bitter Principles of the Seeds.—The seeds that were dried in the sun were ground in a laboratory mill to a coarse powder and analysed. The following results indicate the general composition: Oil 39%, sugars 0·4%, proteins 8·8% and ash 2·9%.

The seed powder (900 g.) was extracted in a soxhlet with light petroleum in order to remove all the oil. The fat-free residue was quite bitter to

the taste. It was subsequently boiled with methylated spirits for about 12 hours, filtered and the extraction repeated a second time with fresh spirits. The combined extracts were distilled on a water-bath almost to dryness under reduced pressure. The solid residue appeared to be a mixture of crystalline and amorphous substances and tasted strongly bitter. When it was extracted repeatedly with hot water a colourless crystalline residue was left behind and the comparatively non-crystalline portion (A) went into solution in water.

The water-insoluble crystalline solid seemed to be a mixture with an indefinite melting point. By repeated extraction with cold alcohol it was separated again into two fractions (B and C). The residue left unextracted (B) was sparingly soluble in alcohol and more easily soluble in acetic acid and acetone. It was purified by boiling with small quantities of alcohol and finally crystallised from glacial acetic acid. Large colourless rectangular prisms and tablets melting at 292–94° (decomp.) were thus obtained. It contained no N, S, halogens or methoxyl groups. (Found: C, 66.6; H, 6.2; molecular weight by Rast's method 458: $C_{26}H_{30}O_8$ requires C, 66.4; H, 6.4 and molecular weight 470.) $[\alpha]_D$ in acetone solution was -110° , in chloroform -113° and in acetylene tetrachloride -112° . The value given by Higby for the alcoholic solution is -106 – 114 whereas Koller and Czerney reported a value of -142° for the solution in methylene chloride. The higher value seems to be in error. The substance was insoluble in cold aqueous potash and dissolved on boiling. From this solution it could be recovered by acidification. It could not be acetylated with sodium acetate and acetic anhydride. It was therefore identified as limonin.

Limonin is unaffected by concentrated hydrochloric acid, but dissolves in strong nitric acid with a pale yellow colour and with concentrated sulphuric acid it produces a remarkably deep red solution, the colour of which disappears on dilution with water. The crystals do not taste, but a solution in very dilute alcohol is very bitter. It does not respond to the test for the flavanone group with magnesium and hydrochloric acid and does not reduce Fehlings solution. Attempts to methylate it with dimethyl sulphate and potash in the presence of methyl alcohol or acetone were unsuccessful.

The fraction (C) which is easily soluble in cold alcohol was recovered by distilling off the solvent. By repeated recrystallisation first from benzene and then from aqueous alcohol, it was obtained as colourless crystals (rhombic and rectangular plates) melting at 258–60° (decomp.) and the mixed melting point with limonin was depressed. (Found: C, 65.9; H, 6.4; $C_{26}H_{30}O_8$ requires C, 66.4; H, 6.4%.) $[\alpha]_D$ in acetone was -108° (Higby

– 112°). It tasted bitter in very dilute alcoholic solution and in all respects agreed with the description of isolimonin. It gave the same reaction with alkali and acids as limonin.

The total yield of the limonin-isolimonin mixture was about 0·6% on the weight of the air-dry seeds of which a very small part was isolimonin.

The water-soluble bitter fraction (A) was isolated by concentrating the solution under reduced pressure to a small bulk and finally removing the last portion of water by evaporation in a vacuum desiccator. The solid residue which was strongly bitter, was obtained pure by repeated crystallisation from water and was identified as naringin. There was considerable loss during the crystallisation and with a view to estimate the quantity present, the amorphous product obtained in another experiment was hydrolysed with dilute sulphuric acid and the sparingly soluble aglucone thereby obtained was dried and weighed. From this naringin was found to be present to the extent of 0·15% on the weight of the air-dry seeds.

Summary

Convenient methods have been worked out for the isolation of the bitter principles of the peels, rags and the seeds of pamparapanas (Indian Shaddock). The first two contain naringin to the extent of 0·13 and 1% respectively whereas in the seeds naringin (0·15%) and limonin (about 0·6%) are found to be the most important bitter components besides small amounts of isolimonin. Some properties of limonin and isolimonin have been studied.

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