

CHEMICAL INVESTIGATION OF INDIAN FRUITS

Part IV. A Note on the Bitter Principle of a Variety of *Citrus limetta*

BY T. R. SESHADRI

(From the Department of Chemistry, Andhra University, Waltair, now at Madras)

Received August 25, 1943

A VARIETY of *Citrus limetta* available in the northern and north-western parts of India during the hot weather has certain peculiar characteristics. It is quite juicy and the juice when carefully sucked out is almost insipid having only a faint sweet taste. But if the fruit should be crushed leading to pressure on the rags, the juice becomes exceedingly bitter. The bitter principle is present mainly in the rags. The thin peel which is rather difficult to remove is not so bitter. The seeds are very small. The external appearance of the fruits is quite similar to that of the sweet variety of *Cirtus limetta* (mussambi) common in Central and Western India and both go by the same common name, Mitta. The rind is yellow to orange in colour and smooth.

A fruit weighs on an average 200 grams and the juice yield is 100 to 120 grams (50 to 60%). The peels constitute 12-15%, the fresh rags 25-30% and the seeds about 0.7% of the fruit. The results of analysis of juice (average of several experiments) is as below: Brix 8.3°, reducing sugars 6.3%, total sugars 6.3%, sucrose nil, acidity 0.01% as citric acid. Thus there is very little acid and though the sugar content is not very low, sucrose is absent and consequently sweetness is poor.

The bitter principle was extracted from the rags by repeated treatment with cold alcohol and the extract concentrated to small bulk. With a view to detect the existence of more than one crystalline entity, the solid matter was isolated in fractions and then purified. By the addition of an equal volume of water to the alcoholic concentrate fraction (I) was obtained. Concentration of the mother-liquor during which most of the residual alcohol was evaporated, yielded fraction (II). The final mother-liquor (M) was separately examined. When fraction (I) was extracted with excess of boiling acetone, most of it dissolved leaving behind a very small quantity of resinous matter. On allowing the solution to concentrate a crystalline solid was obtained (fraction I A). Addition of water to the mother-liquor yielded an amorphous solid (fraction I B). Fraction (II) was also soluble in acetone and from it fractions (II A) and (II B) were obtained.

Fraction (I A) was insoluble in water, sparingly soluble in alcohol and readily soluble in acetone. It was best crystallised from acetic acid from which it came out as colourless rectangular prisms and tablets. The substance charred and burnt when introduced into a flame and left no residue. It melted at $292-94^{\circ}$ with decomposition. When an alcoholic solution of it was treated with magnesium and hydrochloric acid no red or orange colour was developed. A dilute aqueous alcoholic solution was quite bitter; $[\alpha]_D^{30}, -115.1^{\circ}$ in acetone solution. From these properties it seemed to be limonin and this surmise was confirmed by careful comparison with an authentic sample of limonin obtained from the seeds of Indian shaddock¹ and determination of the mixed melting point. Fractions (I B), (II A) and (II B), when subjected to crystallisation, yielded the same product thereby showing that there was only one bitter principle in all of them.

The aqueous mother-liquor (M) was made 7% acid with sulphuric acid and boiled for 2 hours. To start with there was a clear solution and at the end a dark brown resinous solid was found to have separated out. It was almost insoluble in acetone and an alcoholic extract (sparingly soluble in alcohol) gave no colour with magnesium and hydrochloric acid. No crystalline substance could be isolated from it. Thus the absence of naringenin in the resinous matter and of naringin in the aqueous mother-liquor was established.

Consequently the only crystalline bitter principle of the above variety of *Citrus limetta* is limonin. This bitter substance has been formerly found to occur mainly in the seeds of the lemon, oranges and shaddock and it is invariably accompanied by more or less quantities of the closely related compound, isolimonin.^{2,1}

It seems to be now clear that these complex bitter compounds of unknown constitution contribute also to the bitterness of the rags and peels of certain types of oranges. Isolimonin has been found by Higby³ to be the main bitter component of the peels and rags of American Navel oranges and limonin to be present in the pulp of Valencia oranges. The present note records a further case of this kind. The constitution of limonin is quite indefinite. But its formula seems to be definite as $C_{26}H_{30}O_8$. Since the number of carbon atoms is near those of sterols and resinols, its reactions with the special reagents of these groups of compounds has now been studied. With the Liebermann-Burchard reagent it yields a very pale yellow solution. On the other hand, with the Salkowski reagent, the chloroform layer is colourless with a weak bluish violet fluorescence and the sulphuric acid layer first turns

yellowish brown and in the course of a few minutes becomes deep brown red. These characteristics are different from those of sterols and of resinols.

Summary

A variety of *Citrus limetta* has been found to contain limonin as the only bitter principle of the rags. The characteristics of the fruit and its juice are described.

REFERENCES

1. Seshadri and Veeraraghaviah .. *Proc. Ind. Acad. Sci.*, 1940, 11 A, 505.
2. Köller and Czarney *Monatsh*, 1936, 67, 248 ; 1937, 70, 26.
3. Higby *J.A.C.S.*, 1938, 3014.