CLXIII. VITAMINS AND OTHER CONSTITUENTS OF GRAPE-FRUIT RIND.

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A REVIEW of the literature reveals the fact that the grape-fruit (*Citrus decumana*) has been little studied either botanically or as regards its vitamin or other constituents. Zoller [1918], whose work on grape-fruit constituents is the most comprehensive we have been able to discover, reports considerable variations in the constituents and therapeutic activity of the different kinds of grape-fruit which are obtainable. He classifies these fruits into two main divisions. One, the larger, very bitter, non-edible kind is frequently termed the shaddock [Hume, 1901]. The other, smaller, less bitter, edible variety is the fruit usually met with in commerce, and was the one we employed in this investigation.

The bitter principle found in grape-fruit is the glucoside naringin. This occurs in various parts of the plant, but its concentration appears to be highest in the peel. It would appear from the work of de Vry [1866] and Pfeffer [1874] that naringin is specific to the grape-fruit. Moreover, the closely related glucoside hesperidin, characteristic of the orange but which has been found in a large number of other plants [Wander, 1925], is not found in the grape-fruit [Pfeffer, 1874; Will, 1885, 1887; Van Rijn, 1900]. We find that naringin can be extracted from the peel by cold alcohol, even when diluted with water, by boiling water or by dilute alkali, but is precipitated by acid and is hydrolysed by prolonged heating above 80°.

It has been shown by a number of investigators [Dominguez, 1918; Lopez, 1920; Hawkins, 1921] that in grape-fruit during the process of maturing there is some relationship between the acidity, sugar and glucosidal content. For this reason, we thought it of interest to obtain data in regard to these points on the material whose vitamin content was being examined.

PREPARATION OF EXPERIMENTAL MATERIAL.

Fresh ripe West Indian grape-fruit, weighing about 350 g., were washed with cold water, and dried. The flavedo was removed with a sharp knife, taking care to include as little as possible of the albedo. The moist flavedo was transferred at once to a well-stoppered bottle kept in the refrigerator, from which the daily doses were weighed out.

The amount of flavedo obtained from each fruit was from 15 to 20 g., or 4 to 5 % of the total weight. The moisture content, ascertained by drying to constant weight at 40°, varied from 70 to 75 %. The p_H of both flavedo and albedo, determined electrometrically, using a quinhydrone electrode, and colorimetrically, as previously described [Willimott and Wokes, 1926], ranged from 4.3 to 4.6, and of the juice from 5.1 to 5.2.

In preparing the alcoholic extract, the fresh flavedo was chopped into small pieces, weighed immediately into a well-stoppered bottle of non-actinic glass, and covered with 90 % alcohol at the rate of 1 cc. to each gram of rind. This was stored in the refrigerator, away from direct light, and shaken at frequent intervals for 3 weeks. It was then filtered, giving a clear golden liquid with a characteristic smell and a markedly bitter taste, due to the essential oil and naringin present. Tests applied to this alcoholic extract showed that the enzyme peroxidase, which we have shown to be present in considerable amounts in grape-fruit rind, had not been extracted by the solvent. Analysis gave the following results:

p_H	...	...	...	...	...	4.4
Specific gravity at 15°	...	...	...	...	...	0.9497
Total solids	...	...	...	...	...	5.74 %
Reducing sugar	...	...	...	...	...	2.8 %
Total sugar (after hydrolysis at 40°)	...	...	...	...	...	3.6 %

To obtain the oil for feeding to the experimental animals, the fresh rind was air dried for 1 hour at 40°, then put in a stoppered vessel and covered with acetone. This was set aside in the dark and shaken at frequent intervals for a week. It was then filtered, and the residue extracted for another week with dried ether. The mixed acetone and ether extracts were evaporated *in vacuo*, giving a reddish brown oil with an intensely bitter taste. This was kept in a stoppered bottle in the refrigerator. In addition to any vitamins which might be present, it consisted largely of the terpene *d*-limonene, with smaller amounts of citral, α -pinene, geraniol and linalool, and traces of various esters.

FEEDING EXPERIMENTS.

Vitamin A.

Morgan appears to be the only worker who has published a detailed study of the vitamin A content of grape-fruit. In testing the peel [1923], she used a sample prepared by Mr C. P. Wilson at the Research Laboratory of the California Fruit Growers' Exchange. This had been dried at 65 to 70°, and averaged 34 % of the fresh peel, and 8 to 10 % of the whole fresh fruit. In testing it for vitamin A, the curative method was employed. The rats accepted grape-fruit peel less readily than orange or lemon peel, and although 1 g. doses were administered, the whole of it was not eaten. Her finding was that dried outer grape-fruit peel is ineffective as a source of vitamin A. In a later paper

[Morgan and Chaney, 1924], the rind oil was also tested, and a dose as large as 1 g. was found to contain no appreciable amount of vitamin A. In these experiments the supply of vitamin D does not appear to have been controlled. Moreover, Morgan's results are not in accord with the preliminary findings of Cooper [1921], who states, without giving experimental details, that grape-fruit peel contains fat-soluble vitamin. It seemed to us desirable, therefore, in continuing our studies on the *Citrus* fruits, to investigate the vitamin A content of grape-fruit flavedo, taking the precaution emphasised by Drummond, Coward and Handy [1925] that an adequate amount of vitamin D was supplied.

Fig. 1. Vitamin A in grape-fruit flavedo and its 90 % alcoholic extract.

↓ Grape-fruit flavedo, 0.5 g. per head per day.

↓↓ Grape-fruit flavedo 90 % alcoholic extract, 0.5 cc. per head per day.

↓↓↓ Cod-liver oil, 3 drops per head per day.

Three standard piebald rats of 40 to 45 g. weight were restricted to a vitamin A-free diet, employing the usual Hopkins' basal ration of this laboratory. Vitamin D was supplied by direct irradiation of the animals, at a distance of 2 feet from a quartz mercury vapour lamp, for 10 minutes daily. At the end of 4 weeks growth had practically ceased. The fresh flavedo of ripe grape-fruit, finely minced and thoroughly mixed in with the basal ration, was then supplied at the level of 0.5 g. per head per diem. After the initial distaste of the animals had been overcome, practically the whole was eaten. A definite, though temporary, resumption of growth occurred. On the 49th day, when growth had ceased, the rind was substituted by an equivalent quantity (0.5 cc.) of the 90 % alcoholic extract. It was attempted to feed this orally by means of a hypodermic syringe, but the animals evinced strong dislike of the concentrated

bitter extract. Dilution with water had undesirable precipitation effects, besides increasing the amount to be administered. The extract was therefore well mixed in with the basal ration, and careful observation showed that the food intake was not decreased. Growth was resumed, but in the case of two animals proved only temporary, and on the 60th day of the experiment the third animal was also stationary. The three animals were all well below normal weight, and in two of them definite xerophthalmia had already developed. As it had now been clearly established that grape-fruit rind fed at this level was insufficient to protect the animals from xerophthalmia, and to sustain normal growth, cod-liver oil was supplied at the rate of three drops per head per diem. Vigorous growth was promptly resumed, and the xerophthalmia cleared up. On the 68th day of the experiment, when the animals appeared to be rapidly approaching normal, they were chloroformed and autopsied. No definite pathological changes could be observed, and it is worth while noticing that there was an appreciable amount of fatty tissue round the heart, indicating a satisfactory state of nutrition.

The result of this experiment, in which the animals were able to live and even grow slightly for 60 days on a diet in which the sole source of vitamin A was grape-fruit rind, seemed to indicate that the fresh flavedo of the grape-fruit contains small but definite amounts of this vitamin. The amount we had fed was only about a sixth of that given by Morgan, making allowance for the moisture in the fresh rind. In our experience it was impossible to induce the animals to take a larger dose, on account of the bitter taste of the material. It seemed desirable, therefore, to test the fresh rind oil, of which a proportionately larger dose could be given, assuming that the vitamin A is concentrated in the oil in grape-fruit rind, as Morgan and Chaney had shown it to be in orange rind.

Three standard albino rats were restricted to the same vitamin A-free diet. During this preparatory period one died, and its growth is therefore not shown in the figure. On the 29th day, when growth appeared to be held up, the oil extracted from the fresh rind was fed at the level of 0.1 g. per head per diem. A calibrated pipette was used, and as the oil had such a bitter taste that the animals would not tolerate its direct administration, it was mixed in with the basal ration. The food intake remained at about 10 g., and most of the oil was consumed. There was no growth response, and severe xerophthalmia developed in both animals. On the 48th day, when it became obvious that the two animals were suffering from definite vitamin A deficiency, cod-liver oil was fed at the rate of three drops per head per diem. The smaller animal, which was in the more severe condition, was chloroformed three days later. An autopsy showed severe xerophthalmia, enlarged suprarenals, and thinning of the gut. The other animal gradually recovered. Our conclusion is that grape-fruit rind oil contains at most only slight traces of vitamin A.

Fig. 2. Vitamin A of grape-fruit oil.

↓ Rind oil fed 0.1 g. per head per day.

↓ Cod-liver oil fed 3 drops per head per day.

Vitamin B.

Grape-fruit flavedo does not appear to have been previously investigated for vitamin B. In view of the finding of one of us [Willimott, 1926] that lemon flavedo contains considerable amounts of this vitamin, and also of the results reported by Morgan and Chaney [1924] that both orange and lemon peel contain it, it was thought of interest to examine the flavedo of the grape-fruit.

Four female standard albino rats of 54 to 64 g. weight were given a diet deficient in vitamin B. As it has been shown that lemon juice contains vitamin B [Bacharach, 1925], this addendum was omitted. It was not possible for the animals to obtain a supply of vitamin B by consumption of their faeces, as their cages were placed on trays into which the faecal matter fell out of their reach. On the 41st day of the experiment, growth in all four animals had ceased, the average weight being only 78 % of the normal. Evidences of the onset of paralysis were observed. Finely minced fresh grape-fruit flavedo, thoroughly mixed in with the basal ration, was now administered at the level of 0.5 g. per head per day. There was vigorous resumption of growth, and all symptoms of paralysis rapidly disappeared. On the 67th day, when the experiment was terminated, all four animals were in excellent condition, and growing rapidly. The average weight was about 90 % of the normal. From this experiment the conclusion was drawn that grape-fruit flavedo contains considerable amounts of vitamin B.

In view of the previous finding of one of us that 90 % alcohol would extract vitamin B from fresh lemon rind under certain specified conditions, it was thought of interest to test for this vitamin the alcoholic extract which we had prepared. A group of two male and one female rats was put on a vitamin B-free diet as before. After definite vitamin B deficiency had been established, 0.25 cc. per head per day of the alcoholic extract was administered. As this did not produce a marked average resumption of growth, the dose was increased to 0.5 cc., and later to 1.0 cc. The effect on growth was not strongly pronounced,

although no neuritic symptoms could be observed, and at the end of the experiment the animals appeared quite normal. It seemed from this result that the alcohol had not completely extracted the vitamin B from the rind tissue.

Fig. 3. Vitamin B of grape-fruit flavedo.
↓ 0.5 g. flavedo, fed per head per day.

CONCLUSIONS.

The results of our experiments show that grape-fruit rind contains little if any vitamin A, and that it gives a strong peroxidase reaction. Morgan and Chaney [1924], in commenting on the marked contrast in vitamin A content of both peel and juice between the three *Citrus* fruits, orange, lemon and grape-fruit, suggest that this may be related to the character of the lipochromes present. It is possible that light might be thrown on the problem by a study of the reaction and enzyme content of these three

Table I. *Vitamins, oxidising enzymes and reactions in ripe Citrus fruits.*

	Orange <i>Citrus aurantium</i>	Lemon <i>Citrus medica</i> var. <i>Limonum</i>	Grape-fruit <i>Citrus decumana</i>
A. Rind (flavedo)			
Vitamin content A	++	Slight	Very slight
" " B	++	++	++
" " C	+	Very slight	Not yet ascertained
pH	4.4-4.8	4.2-4.4	4.3-4.6
Oxygenase reaction	-	-	-
Peroxidase reaction	+	+	++
B. Juice			
Vitamin content A	++	-	-
" " B	+	+	+
" " C	+++	+++	+++
pH	3.6-4.2	2.4-2.6	5.1-5.2
Oxygenase reaction	-	-	-
Peroxidase reaction	±	+	+

fruits. We have already published data on the reaction and tests for oxidising enzymes in the peel of the lemon at different stages of growth. This work has now been extended to the orange and grape-fruit. Samples of oranges and grape-fruits of different degrees of maturity are being received from California, and examined by the methods previously described [Willimott and Wokes, 1926]. In Table I are summarised data so far obtained by other workers and ourselves bearing on these points.

SUMMARY.

1. Grape-fruit rind contains considerable amounts of vitamin B, but practically no vitamin A. The content of vitamin C has not yet been investigated.
2. Naringin, the specific glucoside of the grape-fruit, was found present in the rind and in alcoholic extracts of it.
3. Data are presented on the reaction and content of oxidising enzymes as compared with the vitamin A content in the three *Citrus* fruits.

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