Phytochemistry, 1968, Vol. 7, pp. 641 to 645. Pergamon Press. Printed in England

CHEMICAL COMPONENTS OF THE FRUITS OF *PSIDIUM GUAVA**

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(*Received* 6 *September* 1967)

Abstract—The fruits of *Psidium guava* were examined at three different stages of growth to follow the chemical changes. From the unripe fruits an ester of hexahydroxydiphenic acid with L-arabinose has been obtained and its constitution established. It disappears in the ripe fruits, which contain mainly free ellagic acid. Leucocyanidin and oxalates are present at their maximum in the unripe fruits and diminish with ripening.

IN PREVIOUS papers[1,2] the polyphenols present in the bark and leaves of *Psidium guava* have been described. The fruits have been extensively studied because of their nutritive value. There are two varieties, pear guava and apple guava. The former usually has pink-coloured flesh, while the latter has not only the shape of an apple but frequently a red skin and usually colourless flesh. The unripe fruit is considered indigestible, while the ripe one is a good aperient. A high percentage of vitamin C, carotene, vitamins B_1, B_2, B_6, free sugars (glucose, fructose and sucrose), a high percentage of pectin and water-soluble araban have been reported to be present in these fruits. The present study relates to polyphenols which may be responsible for the highly astringent taste of the fruits especially when unripe, and has been made at different stages of ripening, the juice and the pulp being examined separately.

Young unripe fruits (3 *weeks old*). The highly acidic juice contained a considerable amount of oxalates, both soluble and insoluble in water, and a small amount of protein but no polyphenols.

Alcoholic extract of pulp. The concentrated alcoholic extract of the pulp was fractionated by solvent extraction. Chlorophyll, waxy matter and carotenoids were removed by light petroleum, and ether extracted quercetin, guaijaverin, free gallic acid and ellagic acid. Subsequent extraction with ethyl acetate gave mainly leucocyanidin along with small amounts of a sugar ester. The remaining extract contained mainly the sugar ester along with some pectin and free sugars. The sugar ester was purified through its lead salt and then by removing mineral matter through ion-exchange resins; finally it was obtained crystalline and found to be chromatographically pure (yield 0·1 per cent). It was weakly laevorotatory, gave blue precipitate with alcoholic ferric chloride and positive Molisch test. On hydrolysis with aqueous sulphuric acid or alkali it gave ellagic acid and arabinose in 1:1 proportion, indicating it to be an ester of hexahydroxydiphenic acid with arabinose, and this was confirmed by quantitative alkali hydrolysis of its acetate and also by u.v. (λ_{max} 272 nm as compared to 252 nm of the O-diglucoside of ellagic acid[1,2] present in stem bark and leaves of this plant) and i.r., ν_{max} at 1740 cm^{-1}.

* A preliminary note on this subject was published in *Current Sci. (India)*, 33, 334 (1964).

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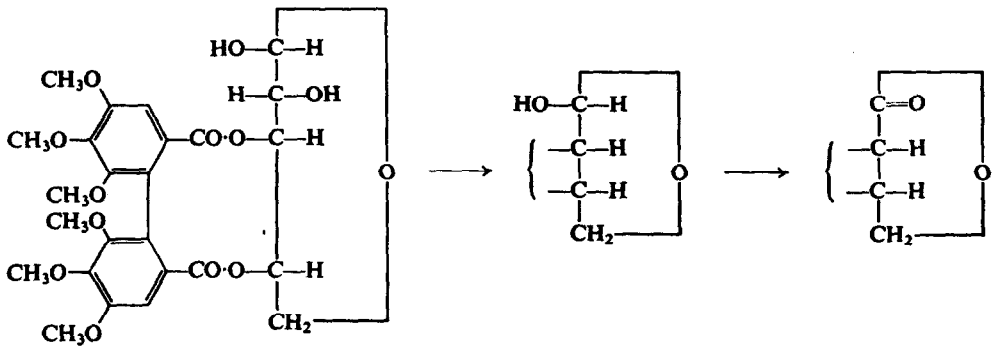
[1] T. R. SESHADRI and K. VASISHTA, *Phytochem.* 4, 317 (1965).

[2] T. R. SESHADRI and K. VASISHTA, *Phytochem.* 4, 989 (1965).

Methylation of the phenolic hydroxyls of the arabinose ester with diazomethane and subsequent hydrolysis gave hexamethoxydiphenic acid in its laevorotatory form. The arabinose ester and its methyl ether gave a brownish purple colour with aniline hydrogen phthalate on paper chromatograms and the ether reduced Fehling's solution in the cold, suggesting that the reducing group in the sugar moiety is free.[3]

The presence of a glycol system in the methylated sugar ester was confirmed by qualitative and quantitative experiments using periodic acid. When the oxidation product was further oxidized with bromine it yielded a stable lactone and not an acid as shown by chemical tests and i.r. spectrum. These results lead to the conclusion that the sugar is in the pyranose form (I).



(I)

Thus the hydrolysable tannin from guava fruits has two novel features: (1) it appears to be the first case where arabinose forms the carbohydrate core; (2) hexahydroxydiphenic acid is linked to two adjacent oxygens on carbon atoms 3 and 4, while in all the other ellagitannins so far known, the links are with either 2 and 4 or 3 and 6 carbon atoms of glucose. This may be due to the fact that in β-glucose, which is the common carbohydrate core of the ellagitannins, neighbouring hydroxyl groups are *trans* to each other while in arabinose hydroxyls on carbon atoms 3 and 4 are *cis*.

Half-grown fruits (*6 weeks old*). The juice contained negligible amount of insoluble oxalates and soluble oxalates were present in much smaller amounts. It contained small amounts of soluble chlorides and sulphates of sodium and potassium and free sugars, glucose, maltose and arabinose in relatively larger quantities. For the extraction of the pulp, a modified water extraction in the cold was used in order to obtain the arabinose ester in a pure state and good yield as well as to prevent the possibility of other changes such as alcoholysis. The arabinose ester thus obtained was identical with the one from the young unripe fruits. However, the yield from the half-ripe fruits was much lower (0·01 per cent). From the pulp left after water extraction, boiling ethanol extracted free ellagic acid (0·13 per cent). This, and the presence of free sugars, including arabinose, indicates that during the ripening process the sugar ester is probably not synthesized.

Ripe fruits. The two varieties of guava, apple and pear guavas, were investigated. The juice of ripe fruits contained almost negligible amount of oxalates but more free sugars. Both varieties were found to contain almost the same amounts of leucocyanidin which is concentrated in the skin and the seeds, the pulp containing very little. No flavone was detected in

[3] L. HOUGH, J. K. N. JONES and W. H. WADMAN, *J. Chem. Soc.*, 1702 (1950).

the ripe fruits, the small amount present in the unripe fruits seeming to have disappeared. Free ellagic acid was isolated from both varieties, 0·2 per cent from the red and 0·05 per cent from the white variety, but no trace of the arabinose ester of hexahydroxydiphenic acid was detected. The skin of the apple guavas very often has red spots, which have been considered to be produced as a result of injuries received: frequently the whole skin is red. This red skin was found to contain a diglycoside of cyanidin, which resembled mecocyanin in its colour reactions.[4]

Changes in polyphenols during ripening. Goldstein and Swain[5] found that there is definite loss of astringency during ripening of fruits and this is connected with the increased polymerization of tannins particularly leucoanthocyanidins. Another process which seems to occur is glycoside hydrolysis, e.g. in grapefruit and related citrus fruits, the water-soluble and bitter naringin, the rhamnoglucoside of naringenin, is found at its maximum in unripe fruits and during ripening hydrolysis produces the aglycone which is devoid of bitterness and sparingly soluble in water.[6]

A similar feature is met with in the guava. The soluble and astringent arabinose ester of hexahydroxydiphenic acid is apparently hydrolysed during ripening and leucocyanidin also diminishes. The disappearance of oxalates and increase of carbohydrates, pectins and vitamins during ripening add to the food value.

EXPERIMENTAL

R_f values relate to circular paper chromatograms, the solvents being: (A) butanol:acetic acid:water (4:1:5) upper layer, (B) *m*-cresol saturated with water, (C) phenol–water upper layer, (D) phenol–water (1:9), (E) butanol saturated with water, (F) butanol saturated with ammonia, (G) acetic acid:water:hydrochloric acid (10:30:3). Ultra-violet spectra were taken in ethanol solution.

Unripe fruits (3 *weeks old*). Fresh fruits (800 g) were crushed in a fruit extractor. The juice (J) (150 ml), which was quite astringent, was saturated with ethyl acetate and kept. The crushed pulp was extracted with ethanol (500 ml, 48 hr × 3) at room temperature, and then with aqueous ethanol (1:3 v/v). The combined extract (2 l.) was concentrated under reduced pressure (200 ml) and, on keeping, some green sticky matter separated out. The clear brown solution (S) was decanted off.

Fruit juice (*J*). The pH of fresh juice was 4·0. On saturation with ethyl acetate, insoluble oxalates, mainly of calcium, separated out. The remaining juice, on evaporation, yielded soluble oxalates of sodium and potassium. The juice turned yellow with concentrated HCl and deep brown with concentrated HNO_3; the colour intensified on adding ammonia, and gave a yellow precipitate with NaOH. It reduced Fehling's solution even in the cold. These tests indicated the presence of proteins and sugars.

Fruit pulp. (1) *Light petroleum extract* (*carotenoids*). The brown aqueous alcoholic concentrate (S) was continuously extracted with light petroleum, which removed most of the chlorophyll, waxy matter and carotenoid compounds. The yellowish green extract gave a deep blue colour with conc. H_2SO_4 indicative of carotenoids.

(2) *Ether extract: (quercetin, guaijaverin, gallic acid, and arabinose ester of hexahydroxydiphenic acid).* The remaining concentrate (S) was continuously extracted with ether for 18 hr. On paper chromatogram (A) the extract gave a green ring for chlorophyll, R_f 1·0, for quercetin and guaijaverin yellow rings, R_f 0·35 and 0·67 respectively (spray, ammonia). With alcoholic $FeCl_3$ spray the latter two rings became green while two more blue rings were obtained with R_f 0·42 and 0·75, corresponding to the arabinose ester of hexahydroxydiphenic acid, described later, and gallic acid; identity was confirmed by comparison with authentic samples. The ether extract gave positive Mg–HCl test for flavonoids and Pew's test for 3-glycosides of flavonols. After hydrolysis with 7 per cent H_2SO_4 guaijaverin ring disappeared and quercetin ring became more intense and further ellagic acid was prominently formed and it could be easily isolated.

Ethyl acetate extract (*leucocyanidin*). The remaining concentrate (S) was continuously extracted with ethyl acetate (10 hr), the yellow extract concentrated under diminished pressure and a small amount of sugar ester that separated was removed, and light petroleum (40–60°) added slowly to separate sticky impurities first and later colourless leucocyanidin. It finally crystallized from ethyl acetate–light petroleum as

[4] R. Robinson and G. M. Robinson, *Biochem. J.* **25**, 1687 (1931).
[5] J. L. Goldstein and T. Swain, *Phytochem.* **2**, 371 (1963).
[6] T. R. Seshadri and J. Veeraraghaviah, *Proc. Indian Acad. Sci. Sect. A* **11**, 505 (1940).

colourless prisms (0·5 g), gave a single ring, R_f 0·3, (B; spray: vanillin–HCl) and green colour with alcoholic ferric chloride. With ethanolic hydrochloric acid it gave cyanidin.

Isolation of the arabinose ester of hexahydroxydiphenic acid. The remaining concentrate (S), on treatment with neutral lead acetate, gave buff-coloured lead salt (filtrate marked F) which was decomposed with H_2S first in ethanol and then water solution. The combined solutions, after concentration, were kept saturated with ether in the cold. The colourless sugar ester separated out along with some mineral matter. Repeated crystallization from moist alcohol gave a fairly pure sample which could be used for most of the experiments. To obtain it absolutely free from mineral matter, its aqueous solution was repeatedly passed through a mixed ion-exchange resin column, till its conductance was constant. It was evaporated in a rotary evaporator and the residue, on crystallization from alcohol and aqueous alcohol, gave pure sugar ester as colourless rectangular plates (0·1 per cent of fresh fruit) decomposing at 230–235°. It gave positive Molisch test, blue precipitate with alcoholic ferric chloride and yellow solution with aqueous alkalis. It was readily soluble in water and sparingly in most of the dry organic solvents except pyridine. It gave a single ring on paper chromatogram, R_f 0·42 (A, spray: alcoholic ferric chloride); $[\alpha]_D^{25}$ −24·2° (*C*, 0·4 in pyridine) (Found: C, 51·0; H, 4·0. Calc. for $C_{19}H_{16}O_{13}$: C, 50·5; H, 3·6 per cent). λ_{max} 272 nm; ν_{max} (kBr) 3550, 1740, 1610, 1575, 1495, 1430, 1350, 1290, 1240, 1160, 1090, 1060, 1040, 915, 895, 795, 775 and 755 cm^{-1}.

Hydrolysis. The sugar ester was refluxed with 7 per cent H_2SO_4 for 2 hr, the solution cooled and the solid separated by centrifuge and washed with water. It did not melt up to 360°, λ_{max} 255 nm, and the colour reactions agreed with authentic ellagic acid. The remaining solution was found to contain arabinose by mixed paper chromatography, R_f 0·66 (D; spray, aniline hydrogen phthalate), and its phenylosazone, m.p. 206°, identical with that of authentic L-arabinose. The proportion of ellagic acid and arabinose was estimated to be 1:1. The arabinose ester on hydrolysis with 5 per cent NaOH yielded ellagic acid and arabinose identified by comparison with authentic samples.

Acetylation of the crude arabinose ester (0·10 g) was done with acetic anhydride (7 ml) and fused sodium acetate (0·2 g) at 130–140° for 2 hr. The acetate crystallized from ethyl acetate–light petroleum as colourless prisms, m.p. 200–202°; it was free from mineral matter (Found: C, 53·3; H, 4·6. $C_{35}H_{32}O_{21}$ required: C, 53·3; H, 4·1 per cent). Quantitative hydrolysis of the acetate was carried out with 5 per cent NaOH. Ellagic acid was weighed and arabinose estimated by Folin and Wu's method. (Found: ellagic acid, 36·8 per cent; arabinose, 17·9 per cent. $C_{35}H_{32}O_{21}$ required: ellagic acid, 38·3 per cent; arabinose, 18·9 per cent).

Methylation and hydrolysis (formation of hexamethoxydiphenic acid). The arabinose ester (0·2 g) in acetone was methylated with CH_2N_2, and the methyl ether was hydrolysed with 5 per cent NaOH (13 ml) at room temperature. The solution was strongly acidified and the solid collected by centrifuge. Some more solid was obtained by extracting the solution with ether. This compound crystallized from ethyl acetate–light petroleum as colourless long needles, m.p. 162° (lit. 161°); $[\alpha]_D^{20}$ −23·0° (*C*, 0·52 in ethyl acetate) and R_f 0·33 (F; spray: bromophenol blue).

Tests for free reducing group. The arabinose ester on paper chromatogram gave a brownish purple ring with R_f 0·48 (D) when sprayed with aniline hydrogen phthalate. Methyl gallate under similar conditions did not develop any colour. Moreover, the sugar ester, after methylation with diazomethane, also gave a deep brown ring with R_f 0·28 (E; spray: aniline hydrogen phthalate); it reduced Fehling's solution in the cold.

Periodic acid oxidation.[7] The methylated arabinose ester gave positive periodic acid test, forming a white precipitate of $AgIO_3$ when treated with acidified $AgNO_3$ in presence of periodic acid. Tetramethyl leucocyanidin was used as a control when a similar white precipitate was obtained. This test confirmed the presence of a glycol unit in the molecule.

For quantitative estimation, the diazomethane methylated arabinose ester (5·84 mg) was dissolved in ethanol (5 ml) and to it $NaIO_4$ solution (5 ml, 0·0194 M) was added and mixture kept at room temperature (20°) for 24 hr. The amount of $NaIO_4$ consumed was found by titration to be 1·18 moles for 1 mole of the compound.

Bromine oxidation (sugar lactone). The methylated arabinose ester (20 mg) was oxidized with $NaIO_4$ (10 ml, 0·02 M) as above and after removing alcohol the product was extracted with ether, solvent evaporated and residue taken up in aqueous ethanol (15 ml; 50 per cent). Br_2 in $CHCl_3$ was added to it in excess. It was left overnight, the excess of Br_2 removed by aeration and the solution extracted with ethyl acetate; from the dried ($MgSO_4$) solution, a colourless compound crystallized out on adding light petroleum (40–60°). This compound on paper chromatogram did not develop with bromophenol blue but gave a deep brown spot on spraying with alkaline N-hydroxylamine hydrochloride followed by 2 per cent $FeCl_3$ in 1 per cent aqueous HCl (lactone reagent). Its i.r. spectrum in KBr showed the following main peaks: 3440, 2950, 1750, 1731, 1600, 1575, 1470, 1400, 1340, 1210, 1175, 1110, 1030 and 975 cm^{-1} (Found: C, 56·5; H, 5·0. Calc. for $C_{24}H_{24}O_{12}$: C, 57·1; H, 4·8 per cent.

Free sugars (glucose, maltose and arabinose). The aqueous filtrate (F) from the lead salt, was deleaded with H_2S and concentrated to a brown syrup which gave positive Molisch test and reduced Fehling's solution. Sugars were identified as glucose, maltose and arabinose by paper chromatography and by making their

[7] A. I. VOGEL, *Practical Organic Chemistry*, 3rd edn., p. 1070, Longmans, London (1956).

phenylosazones, which took different times to appear. Estimation showed 72 mg of reducing sugar (as glucose) per 100 g of the fresh fruit.

Half-ripe (1½-month-old fruits). The fruit juice was very astringent, pH 4·5, had negligible amount of insoluble oxalates and proteins while soluble oxalates as well as chlorides and sulphates of sodium and potassium were present in appreciable amounts. Free sugars, glucose, maltose and arabinose were also detected.

Fruit pulp (arabinose ester of hexahydroxydiphenic acid and ellagic acid). The pulp from the half-ripe fruits (150 g) was extracted with water (200 ml) at 10° (3 × 10 days). Purification was done by the lead salt method, and from the solid thus obtained flavonoids and free gallic acid were removed by ether extraction. The ether extract on paper chromatogram gave three definite rings, R_f 0·35 (quercetin), 0·67 (guaijaverin) and 0·75 (gallic acid) (A; spray, alcoholic ferric chloride). The ethyl acetate removed a small amount of leucocyanidin. The residue crystallized from boiling moist ethanol (1:9; v/v) as big colourless prisms (15 mg; 0·01 per cent), m.p. 230–232° (decomp.), R_f 0·42 (A), identical with the arabinose ester isolated from 3-week-old fruits, described earlier. The fruit pulp on further extraction with boiling ethanol yielded ellagic acid in 0·13 per cent yield.

Ripe fruits (250 g) were used. The juice (pH 4·8) contained pectins and polysaccharides, and negligible amount of oxalates, gave positive tests for Cl^- and SO_4^{2-} and had considerable amount of free sugars (shown by paper chromatography).

The pulp along with skin and seeds was extracted with ethanol at room temperature and the extract fractionated by solvent extraction. Chlorophyll, waxy matter and carotenoids were removed by light petroleum. Ether yielded only a little ellagic acid (15 mg). Ethyl acetate extract on concentration deposited ellagic acid (25 mg) and the solution contained mainly leucocyanidin (50 mg). The aqueous mother liquor on concentration gave more ellagic acid (90 mg).

Anthocyanin from the red skin of apple guavas. The deep-red skin from four ripe fruits was peeled and extracted with cold 2 per cent alcoholic HCl (30 ml × 3), and to the combined extract excess of ether was added, when a deep-red aqueous acid layer separated out. It was washed with ether and ethyl acetate. The anthocyanin was not extractable with amyl alcohol. It gave blue colour with NaOH and blue violet with Na_2CO_3 and NaAc solutions. After acid hydrolysis, the aglucone was purified by partition between amyl alcohol and 1 per cent aqueous HCl and finally by paper chromatography. It then agreed fully with cyanidin; R_f 0·36 (G); λ_{max} 0·1 per cent EtOH–HCl 540 nm. The anthocyanin was therefore a diglucoside of cyanidin and most probably mecocyanin.[4]

Flowers. The petals of a few flowers were extracted with alcohol in the cold. The light-yellow extract gave reactions for the presence of flavonoids and leucoanthocyanidins and on paper chromatogram gave two deep-yellow rings with R_f 0·35 and 0·67 (A; spray: ammonia) which corresponded with quercetin and its 3-arabinoside, guaijaverin. Leucocyanidin was identified by conversion into cyanidin. Gallic or hexahydroxydiphenic acid derivatives were absent.

Acknowledgement—We convey thanks to C.S.I.R. (India) for the grant of Fellowship to one of us (K. M.).