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POLYPHENOLS OF THE STEM BARK OF *PSIDIUM GUAVA*
THE CONSTITUTION OF A NEW ELLAGIC ACID GLYCOSIDE (AMRITOSIDE)

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Abstract—From the ethanolic extract of the fresh inner stem bark of *Psidium guava* were isolated ellagic acid, and another acid which may be luteic or hexahydroxy-diphenic acid in low concentration, and in larger amounts a new glycoside of ellagic acid, now named amritoside, and leucocyanidin. Amritoside has been shown to be the 4β-gentiobioside of ellagic acid.

INTRODUCTION

AS INDICATED in an earlier note,[1] the stem bark of *Psidium guava* is very astringent and is used for tanning and dyeing in certain parts of India. This led us to investigate its chemistry.

Leucocyanidin

The fresh inner stem bark was extracted with alcohol in the cold and the solution obtained fractionated by solvent extraction. Light petroleum extracted some waxy matter along with carotenoids. Subsequent continuous extraction with ether gave a small amount of carotenoids along with traces of amritoside, a diglucoside of ellagic acid, described later. Ethyl acetate extracted leucoanthocyanidin in quite good yield (0·4 per cent); the product obtained by boiling with alcoholic hydrochloric acid was identified as cyanidin by colour reactions, paper chromatography and absorption maximum. The acetate, methyl ether and methyl ether acetate derivatives of the leucocyanidin were prepared and characterized as described before.[2,3] Two methyl ethers having different melting points, solubilities and optical rotations were actually obtained, but both gave the same tetra-methylcyanidin on boiling with ethanolic hydrochloric acid. Similar results have been obtained with leucocyanidin isolated from other parts of this plant, and also with the substance from other sources.[2,3] The presence of stereoisomeric mixtures explain this feature.

Ellagic Acid and its Glycoside, Amritoside

On standing, the aqueous alcoholic mother liquor, after extraction with ethyl acetate, deposited an almost colourless solid which was found to be a mixture containing a very small amount of ellagic acid and a major amount of its glycoside. The two could readily be separated by taking advantage of the water solubility of the latter. On completely evaporating the aqueous alcoholic mother liquor over potash, a residue was obtained which gave further quantities of the colourless glycoside by crystallization from aqueous ethanol. From the mother liquor of this crystallization further amounts of glycoside could be isolated by forming the lead salt and decomposing it with hydrogen sulphide. A small amount of a second colourless substance was also isolated from the lead salt which was found by paper chromatography

[1] T. R. SESHADRI and K. VASISHTA, *Curr. Sci.* (*India*) **32**, 499 (1963).
[2] G. R. NAGARAJAN and T. R. SESHADRI, *J. Sci. Ind. Res.* (*India*) **20B**, 615 (1961).
[3] J. S. CHADHA and T. R. SESHADRI, *Curr. Sci.* (*India*) **31**, 56 (1962).

to be a mixture containing a small quantity of ellagic acid. The major compound appears to be the corresponding mono- or di-carboxylic acid. The higher u.v. absorption maximum (266 mμ) of the mixture as compared to that of ellagic acid (255 mμ) suggested the presence of more hydroxyl groups. Elemental analysis and the ready conversion into ellagic acid by boiling ethanolic hydrochloric acid, provided further support for the above conclusion. A strong peak at 1690 cm^{-1} in the i.r. spectrum confirmed the presence of a free carboxyl group, and an inflection at 1750 cm^{-1} for the lactone ring may be due to the presence of ellagic acid or luteic acid in the mixture.

Constitution of Amritoside

Since it is a new compound and the first glycoside of ellagic acid, it is named amritoside (derived from the Indian name of the plant "amrit"). The glycoside (0·1 per cent of the fresh bark) had many properties similar to ellagic acid giving a bluish-green precipitate with ferric chloride and a yellow solution with alkalis; it had a similar R_f value on paper chromatography in several different solvents. The u.v. absorption curves of the two were similar with a small difference of 3 mμ in the absorption maximum, λ_{max} for the glycoside being at 252 mμ; the logϵ values were, however, different. Infrared spectra of the two were also similar with a strong carbonyl band at 1750 cm^{-1}, indicating the presence of an α,β unsaturated lactone.[4] The glycoside was readily soluble in water and moderately so in hot, partially aqueous organic solvents such as alcohol and ethyl acetate, whereas ellagic acid was sparingly soluble. The glycoside gave a positive Molisch test for carbohydrate, melted with decomposition and decomposed at 248–250° (cf. ellagic acid, m.p. > 360°).

On hydrolysis with aqueous sulphuric acid amritoside gave ellagic acid and glucose. Ellagic acid was unequivocally identified by colour tests, solubility, mixed paper chromatography, u.v. and i.r. spectra (1750 cm^{-1} for dilactone) and potentiometric titration by comparison with an authentic sample.[5,6] The lactone rings did not open on potentiometric titration. Two out of the four hydroxyl groups present in the molecule reacted first, probably the two more strongly acidic groups at the 3- and 3′-positions. The remaining two hydroxyls are more weakly acidic and react subsequently. The sugar was identified as glucose by paper chromatography and preparation of phenylosazone. Quantitative studies of the products suggested that the compound was a diglucoside and this was confirmed by the elemental analysis of its methyl ether and acetate, as well as the acetoxyl estimation of the latter.

The fact that amritoside was unaffected by aqueous alkali eliminated the possibility that it was an ester involving glucose and hexahydroxydiphenic acid. The glycoside did not give positive colour with aniline hydrogen phthalate reagent when sprayed on paper chromatograms, showing that there is no potential aldehydic group free in the sugar moiety.

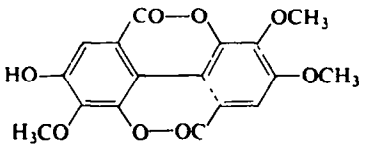
Position of Sugar Units

The position of the two glucose units was determined with the help of: (*a*) spectral shifts, (*b*) potentiometric titration, and (*c*) complete methylation and hydrolysis of the glucoside. It was noted by Jurd[7,8] that the strongly acidic groups in the 3,3′-positions of ellagic acid are ionized by both sodium acetate and sodium ethylate, but hydroxyls located in the 4- and

[4] L. J. BELLAMY, *The Infra-red Spectra of Complex Molecules* (2nd Ed.), p. 178, Methuen, London (1958).
[5] A. G. PERKIN and M. NIERENSTEIN, *J. Chem. Soc.* **87**, 1412 (1905).
[6] D. E. HATHWAY, *Nature* **177**, 747 (1956).
[7] L. JURD, *J. Am. Chem. Soc.* **81**, 4610 (1959).
[8] L. JURD, K. J. PALMER, F. STITT and J. N. SHOOLERY, *J. Am Chem. Soc.* **81**, 4620 (1959).

4'-positions are weakly acidic and can be ionized only by sodium ethylate. Bathochromic shifts with these two reagents was utilized by Briggs and co-workers[9] to locate the position of a free hydroxyl group in the trimethyl ellagic acid isolated by them from the bark and wood of *Eugenia maire*. Row *et al.*[10] established the positions for methoxyl groups at 3 and 3' in the 3,3'-dimethyl ellagic acid 4-glucoside isolated by them from the wood of *Terminalia paniculata*. In the present study of amritoside, the spectral shifts were measured by the method of Jurd and Horowitz[11] and definite bathochromic shifts were obtained both with sodium acetate and sodium ethylate, indicating that at least one hydroxyl in the 3- or 3'-position is definitely free. In potentiometric titration amritoside behaved exactly like ellagic acid, showing two strong acidic hydroxyl groups. This showed that hydroxyls at the 3- and 3'-positions of ellegic acid are not involved in glucoside formation.

The position of the sugar moiety was finally established by methylation and acid hydrolysis of the glucoside, when a trimethyl ether of ellagic acid was obtained which yielded a monoacetate. This agreed in its properties with 3,3',4'-trimethyl ether of ellagic acid (I). The identity was confirmed by comparison with an authentic sample kindly supplied by Prof. Briggs. The u.v. absorption curve and i.r. spectra of the two were superimposable; the mixed m.p. was undepressed.



(I)

Nature of Glucosidic Links

In order to find out the nature of the two glycosidic linkages, i.e. one between the two glucose units and the other between the disaccharide unit and ellagic acid moiety, hydrolysis of the glucoside with Taka diastase and emulsin was studied. It was unaffected by Taka diastase but underwent complete hydrolysis with emulsin to give 2 moles of glucose per mole of ellagic acid. Thus the nature of the linkages in both the disaccharide and to the aglycone was confirmed as β, and the comparatively low rotation of the glycoside i.e. $[\alpha]_D^{25} - 41{\cdot}4$ (pyridine) is in conformity with its being a β-glucoside. Hence the probability is that it is either a gentiobioside or a cellobioside, laminaribioside or sophoroside. The first seems to be more probable as gentiobiosides occur more frequently in nature as compared to the others.

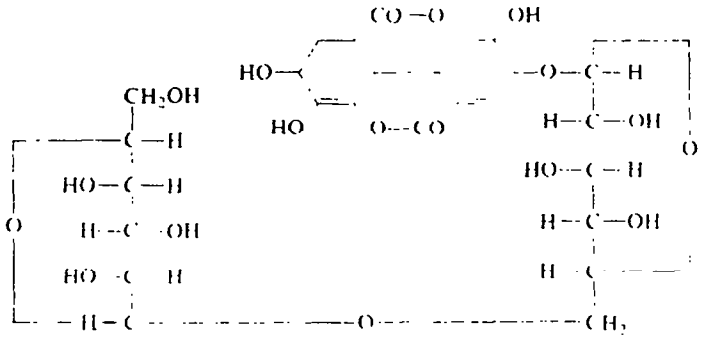
Nature of the Disaccharide

In order to establish the exact nature of the carbohydrate unit, all the phenolic hydroxyl groups of the glycoside were methylated by means of diazomethane and the product was subjected to quantitative oxidation with periodic acid. The consumption of 4 moles of periodic acid with the consequent formation of 2 moles of formic acid, definitely confirmed the identity of sugar unit as gentiobiose. It may be emphasized that this result agrees only if the disaccharide is gentiobiose in the pyranose form (see Formula II). Although there have been several examples of ellagic acid occurring in nature in combination with glucose in

[9] L. H. Briggs, R. C. Cambie, J. B. Lowry and R. N. Seelye, *J. Chem. Soc.* 642 (1961).
[10] L. R. Row and G. S. R. S. Rao, *Tetrahedron* **18**, 357 (1962).
[11] L. Jurd and R. M. Horowitz, *J. Org. Chem.* **22**, 1618 (1957).

what are known as ellagitannins, glycosides of ellagic acid seem to be rare and no gentiobiosides have hitherto been reported.

Ellagic acid occurs widely in nature,[6,12] especially in the Myrtaceae to which *Psidium guava* belongs. Ellagic acid occurs both free and in the form of complex glucose esters of the corresponding unlactonized acid, known as ellagitannins. Partial methyl ethers of ellagic acid have also been reported. Its 3,3'-dimethyl ether has been isolated from the roots of *Euphorbia formosanum*,[13] and Briggs and co-workers[9] have isolated 3,3',4'-trimethyl ellagic



(II)

acid (I) from the bark and wood of *Eugenia maire*. The 4-glucoside of 3,3'-dimethyl ellagic acid has been reported to occur in the heart-wood of *Terminalia paniculata*,[10] and recently Cain[14] has reported the presence of 3-methyl, 3,3'-dimethyl and 3,3',4'-trimethyl ellagic acids in the bark, leaves and wood of *Leptospermum scoparium*.

Biogenesis of the Amritoside (II)

In the biogenesis of glycosides it has been generally considered that the sugar groups enter the molecule at the last stage, though this may not be invariably true. In the present case the location of the sugar unit in the 4-position of ellagic acid may require explanation. As already mentioned there is marked difference in the acidity of the hydroxyls present in the 3,3'- and 4,4'-positions. In methylation the more acidic 3,3'-hydroxyl groups are the first attacked, somewhat analogous to facile ester formation. On the other hand, acyl groups are known to attack less-acidic hydroxyl groups to form more stable acyl esters. This point has been fully established in the acylation of gallic acid where the *m*-hydroxyl is the most favoured.

In ellagic acid, therefore, the 4,4'-positions would be expected to be the most favoured for acylation. The fact that the tetracetate of ellagic acid can be easily deacetylated in the 3,3'-positions to give the stable 4,4'-diacetate by simply boiling its pyridine solution with water,[15] supports such a contention. Glycoside formation seems to fall between these two types of reactions and consequently, although we might expect the formation of 4-glycosides of ellagic acid, it is by no means certain. There is, however, another point to consider in this connexion, and that relates to steric hindrance. It seems probable that a large-sized group like a gentiobiose unit might find it difficult to enter the hindered 3-position. Obviously the formation of the 4-diglucoside is favoured by a combination of these two factors.

[12] E. C. BATESMITH, *Chem. & Ind. B.I.F. Rev.* **R.-32** (1956).
[13] H. SHINODA and C. P. KUN, *J. Pharm. Soc.* **51**, 50 (1931).
[14] B. F. CAIN, *New Zealand J. Sci. Technol.* **6**, 264 (1963); *Chem. Abstr.* 9085(c) (1963).
[15] L. JURD, *J. Am. Chem. Soc.* **81**, 4606 (1959).

EXPERIMENTAL

R_f values relate to circular paper chromatograms, the solvents being: (A) butanol:acetic acid:water (4:1:5) lower layer; (B) butanol saturated with ammonia; (C) *m*-cresol saturated with water; (D) phenol–water upper layer; (E) phenol:water (9:1); and (F) ethanol:water:ammonia (20:4:1). Ultraviolet spectra were taken in ethanol, visible in 0·1% ethanolic hydrochloric acid, and i.r. spectra in KBr discs.

Extraction of the Stem Bark of Psidium guava

The inner fresh stem bark (500 g) was cut into small pieces and extracted with 70% ethanol at room temperature (29°) (3 × 24 hr). The combined extract (3 × 2·0 l.) was concentrated under reduced pressure and the concentrated solution (200 ml) was diluted with water (100 ml). It was continuously extracted with light petroleum when chlorophyll, waxy matter and carotenoids were removed.

Ether Extract (Traces of Chlorophyll, Carotenoids and Amritoside)

The clear, brown residual extract was continuously extracted with ether for 60 hr. The pale-yellow ether extract gave no tests for flavonoids. On paper chromatograms (solvent A) it gave two rings, one visibly yellow and travelling with the solvent front, the other, with R_f 0·40, visible only on spraying with ammonia. The ether was removed and the residue extracted with light petroleum. The petroleum-soluble fraction contained only chlorophyll and carotenoids. The light petroleum-insoluble fraction (5 mg) gave R_f 0·40 (solvent A) and 0·32 (solvent B). Ammonia, alcoholic ferric chloride or bromophenol blue were used as spraying reagents, when the ring developed yellow, bluish-green and pink colours respectively, showing that the substance is a phenolic acid. It gave positive tests for ellagic acid, i.e. bluish-green precipitate with ferric chloride and yellow solution with ammonia, sodium bicarbonate, carbonate and hydroxide solutions. But as it was readily soluble in water it appeared to be a glycoside of ellagic acid. It was extractable with moist ether but after separation did not dissolve in it.

Ethyl Acetate Extract (Leucocyanidin)

The residual aqueous extract from above was continuously extracted with ethyl acetate for 50 hr. The ethyl acetate extract on concentration and keeping in the cold deposited a minor amount of ellagic acid glucoside (10 mg) which was separated. To the solution light petroleum (40–60°) was added when a colourless sticky substance separated out. This crystallized from ethyl acetate–light petroleum mixture yielding colourless prisms (R_f 0·39 solvent C; spray; hydrochloric acid solution of vanillin), yield 2 g.

The leucocyanidin (0·02 g) was refluxed with 10% ethanolic hydrochloric acid (10 ml) for 2 hr. The anthocyanidin thus obtained was purified by solution in amyl alcohol and expulsion into 1% aqueous hydrochloric acid layer by adding excess of light petroleum. It gave all the colour tests of cyanidin and had R_f 0·72 (solvent D) and λ_{max} at 540 mμ, similar to authentic cyanidin.

Leucocyanidin (0·2 g) was acetylated with acetic anhydride and pyridine at room temperature for 48 hr. The acetate crystallized from ethyl acetate–light petroleum mixture as colourless prisms, m.p. 180–183°; yield, 0·28 g; $[\alpha]_D^{30}$ +74·7 (ethyl acetate). (Found: C, 58·4; H, 5·0. Calc. for $C_{27}H_{26}O_{13}$: C, 58·2; H, 4·7%.)

Leucocyanidin (0·40 g) in methanol solution (20 ml) was treated with ethereal solution of

diazomethane in excess and kept in the refrigerator overnight when a colourless solid (prisms) separated out (M-1), which was washed with ether repeatedly (0·17 g). The solution was decanted and excess of diazomethane was destroyed by adding a few drops of glacial acetic acid. The solvents were distilled off completely and residue (M-2) was crystallized from methanol yielding small cream-coloured prisms (0·25 g).

The first methyl ether (M-1) was sparingly soluble in ethyl acetate, methanol, ethanol, chloroform, dioxan, acetone and dimethyl formamide. It was fairly soluble in warm pyridine. On boiling it with 5% ethanolic hydrochloric acid sufficient pink colour developed only after refluxing for 1 hr. For complete conversion into tetramethyl cyanidin (identified by co-chromatography and λ_{max} 535 mμ) longer boiling for 2½ hr was required. It melted at 270°, $[\alpha]_D^{30}$ +90·6 (pyridine). (Found: C, 58·4; H, 5·9. Calc. for $C_{19}H_{22}O_7$: 1½H_2O: C, 58·6; H, 6·4%.)

M-2 was soluble in ethyl acetate, methanol, ethanol and acetone, m.p. 159–160°, λ_{max} 530 mμ, $[\alpha]_D^{30}$ +25·7 (ethyl acetate). (Found: C, 62·9; H, 5·8. Calc. for $C_{19}H_{22}O_7$: C, 62·9; H, 6·0%.) It gave methyl cyanidin readily, even on keeping it at room temperature with 5% ethanolic hydrochloric acid, but for complete conversion refluxing for 1 hr was required.

Methyl Ether Acetate

M-2 (0·01 g) was acetylated with acetic anydride and pyridine at room temperature (48 hr). The acetate obtained crystallized from ethyl acetate–light petroleum mixture as colourless small prisms, m.p. 152–153°; yield 0·015 g. (Found: C, 61·9; H, 5·9. Calc. for $C_{23}H_{26}O_9$: C, 61·8; H, 5·8%.)

Aqueous Alcoholic Mother Liquor (Ellagic Acid and Amritoside)

The residual aqueous extract was kept saturated with ethyl acetate in the cold for 15 days. A brown crystalline solid separated out. It was filtered and washed with small amounts of water (0·02 g) and crystallized from alcohol containing a trace of pyridine as colourless long needles, m.p. above 360°. It had bluish-green colour with ferric chloride, yellow with alkalis and positive Greissmayer's test, i.e. a blood-red colour on treatment with nitric acid containing some nitrous acid. It had R_f 0·3 (solvent B, spray: bromophenol blue) and R_f 0·35 (solvent A, spray: ammonia). Mixed chromatography with an authentic sample of ellagic acid under similar conditions gave a single ring with the same R_f values.

The filtrate and washings from this separation were completely evaporated over potash at room temperature and the residue boiled with alcohol (5 × 30 ml). The solutions were decanted and kept separately. After keeping for 24 hr at room temperature (29°), a compound crystallized from the first four fractions as colourless rectangular plates, but nothing was obtained from the fifth extract. Since the first fraction was contaminated with some brown-coloured impurity, the pure substance was obtained from the other three fractions by decanting off the solvent and washing the crystals with small amounts of dry alcohol. The solid from the first fraction was combined with the original residue left after boiling with alcohol and the total solid was again boiled with small amounts of 80% alcohol (4 × 25 ml). All the four fractions yielded big colourless rectangular plates. The compound obtained, amritoside, was readily soluble in water, moderately in boiling ethanol but sparingly soluble in cold ethanol, ether and ethyl acetate. It dissolved in aqueous sodium bicarbonate, carbonate and hydroxide solutions to give a deep-yellow solution. It gave a bluish-green precipitate with ferric chloride and a positive Molisch test. On heating it turned brown at 248–250°

(decomp.) but did not melt at 360°. It gave a single pink ring in paper chromatography, R_f 0·32 (solvent B, spray: bromo phenol blue). It was markedly astringent; total yield, 0·4 g; $[\alpha]_D^{25} - 41{\cdot}4$ (pyridine). (Found: C, 50·1; H, 4·1. Calc. for $C_{26}H_{26}O_{18}$: C, 49·9; H, 4·2%.)

The mother liquors showed on paper chromatography the presence of a dark-coloured impurity of R_f 0 along with some ellagic acid and amritoside. Attempts at purification by using cellulose or neutral alumina columns were not successful. Saturated aqueous solution of neutral lead acetate was therefore added to the solution, when a cream-coloured lead salt separated out. The salt was filtered, suspended in alcohol and decomposed by hydrogen sulphide. This process was repeated three times and the combined yellow filtrate (500 ml) was concentrated under diminished pressure (40 ml). The remaining lead salt was finally suspended in water and treated again. This process was repeated a number of times in order to extract out all the water-soluble components. The combined aqueous filtrate (0·5 l.) was concentrated on a water bath (25 ml). Both concentrated solutions, on keeping, deposited some brown impurity which was removed and the solutions were saturated with ether and kept in the cold when colourless crystals separated out. The solution from the alcohol decomposition was decanted; the solid obtained was partly soluble in water, the soluble portion was mixed with the aqueous concentrate, and the solution completely evaporated on a water bath and the residue was crystallized from 80% alcohol yielding rectangular plates (15 mg) 248–250° (decomp.). This solid gave all the colour tests for amritoside.

The ether solution was evaporated completely and the residue crystallized from ethanol as colourless plates. In paper chromatography (solvent A, spray: ammonia) it showed the presence of two compounds, one with R_f 0·35, which corresponded to ellagic acid, and the other with R_f 0·56. The mixture gave blue precipitate with ferric chloride, yellow solution with alkalis, positive Greissmayer's test but gave no Molisch test. It did not melt below 300°. Attempts to separate the two entities with ethanol or boiling water were not successful. It gave λ_{max} at 266 mμ in the u.v. and a definite peak in the i.r. at 1690 cm^{-1} indicating the presence of a free carboxyl group and an inflection at 1725 cm^{-1} for a lactone ring. When the mixture (10 mg) was refluxed with 50% alcoholic hydrochloric acid solution (8 ml) for 3 hr, the product obtained was pure ellagic acid. Therefore this substance may be the mono- or di-carboxylic acid with one or both lactone rings of ellagic acid open.

Properties of Amritoside

(1) *Spectra.* Ultra violet, λ_{max} 252 mμ (log ϵ 5·17), similar to that of ellagic acid, λ_{max} 255 mμ (log ϵ 4·87). A definite bathochromic shift was obtained in ethanol containing sodium acetate (20 mμ) as well as with sodium ethylate (42 mμ).

Infra red gave the following main peaks: 3500 cm^{-1}, 3320 cm^{-1} (OH groups), 1750 cm^{-1} and 1720 cm^{-1} (lactone rings), 1625 cm^{-1}, 1560 cm^{-1}, 1550 cm^{-1}, 1520 cm^{-1} (aromatic rings), 1450 cm^{-1}, 1340 cm^{-1}, 1200 cm^{-1}, 1110 cm^{-1} and 1045 cm^{-1}.

(2) *Acid Hydrolysis.* The glycoside (0·25 g) was hydrolysed by refluxing with 7% sulphuric acid (30 ml) for 2 hr on a water bath. After about 25 min a colourless solid separated out from the clear solution. After 2 hr the solution was cooled and the crystalline solid centrifuged off; yield, 0·12 g (48·0 per cent), ellagic acid diglucoside required 48·2 per cent. It crystallized from alcohol, containing a trace of pyridine, as colourless needles, m.p. above 360°. Colour tests and paper chromatography showed it to be ellagic acid. In u.v. λ_{max} was at 255 mμ and the absorption curve was identical with that of synthetic ellagic acid. Infrared spectrum showed the following main peaks: 3550 cm^{-1} (OH group), 1740 cm^{-1},

1720 cm^{-1} (lactone rings), 1620 cm^{-1} and 1520 cm^{-1} (aromatic rings), 1430 cm^{-1}, 1360 cm^{-1}, 1220 cm^{-1} (w), 1190 cm^{-1} (w), 1100 cm^{-1}, 1060 cm^{-1}, 920 cm^{-1} and 758 cm^{-1}.

The acid solution left after removal of ellagic acid was neutralized with barium carbonate and evaporated on a water bath. The brown syrup obtained gave a single brown ring on paper chromatography, R_f 0·58 (solvent E, spray; aniline hydrogen phthalate); authentic glucose under similar conditions gave the same R_f value. The sugar syrup gave a phenylosazone which crystallized as long yellow needles, identical to the phenylosazone of glucose, m.p. 200–202° (lit. 205°).

In a quantitative experiment the sugar solution was rendered alkaline and glucose was estimated by Folin and Wu's colorimetric method (yield 57·2 per cent; diglucoside required 57·3 per cent).

Acetate of Amritoside

The diglucoside (0·05 g) was heated with acetic anhydride (5 ml) and fused sodium acetate (0·05 g) at 130–140° for 2½ hr. The acetate crystallized from ethyl acetate–light petroleum mixture as colourless plates, m.p. 208–210° (70 mg) $[\alpha]_D^{25°}$ +91·6 (ethylacetate). (Found: C, 53·0; H, 4·8; $COCH_3$, 40·0. Calc. for $C_{46}H_{46}O_{28}$: C, 52·7; H, 4·4; $COCH_3$, 41·1%.)

Methylation and Hydrolysis

Amritoside (0·1 g) was taken up in dimethly formamide (8 ml) and to it was added an ethereal solution (50 ml) of diazomethane (10 equivalents). Methanol (20 ml) was subsequently added to make the solution homogenous. It was kept for 24 hr in the cold, the excess of diazomethane decomposed with a few drops of acetic acid and the solvents distilled off under diminished pressure when a sticky residue was obtained. It was washed with ether. On triturating it with methanol (5 ml), a white crystalline solid (0·12 g) separated. It crystallized from ethylacetate as cream-coloured rectangular prisms, m.p. 232–234°C (decomp.). (Found: C, 52·1; H, 5·1. Calc. for $C_{29}H_{32}O_{18}$: C, 52·1; H, 4·8%.)

The amritoside methyl ether (80 mg) was refluxed with aqueous 7% sulphuric acid (10 ml) for 2 hr at 100°. After approximately 20 min a colourless solid separated out. After 2 hr the solution was cooled, the solid that separated (12 mg) was centifuged off, washed with water and the solution along with the washings was continuously extracted with ether for 15 hr. On complete evaporation of ether a further amount of amorphous solid (20 mg) was obtained. The combined methyl ether (32 mg) was finally crystallized from dioxane–petroleum ether mixture yielding long rectangular plates, m.p. 288–289°. Mixed m.p. with an authentic sample of 3,3',4'-trimethyl ellagic acid was undepressed. It gave no characteristic colour with alcoholic ferric chloride, deep yellow solution with alkalis and a single yellow ring on paper chromatogram having R_f 0·4 at 29° (solvent F) alone or mixed with authentic 3,3',4'-trimethyl ellagic acid. (Found: C, 58·6; H, 4·0; OCH_3, 25·0. Calc. for $C_{17}H_{12}O_8$: C, 59·1; H, 3·5; OCH_3 27·0%.) Spectral data: u.v. λ_{max} 246 mμ. In ethanol containing sodium acetate there was no bathochromic shift. The i.r. was superimposable on that of 3,3',4'-trimethyl ellagic acid. The main peaks are 3500 cm^{-1} (OH group), 1750 cm^{-1} and 1725 cm^{-1} (lactone rings), 1600 cm^{-1} (aromatic ring), 1400 cm^{-1}, 1350 cm^{-1} (OCH_3), 1320 cm^{-1} (OCH_3), 1285 cm^{-1} (OCH_3), 1245 cm^{-1}, 1110 cm^{-1}, 1080 cm^{-1}, 980 cm^{-1} and 910 cm^{-1}.

Trimethyl Ellagic Acid Acetate

The trimethyl ether was acetylated with acetic anhydride (3 ml) and pyridine (4 drops) at 29° for 48 hr. The solid obtained crystallized from dioxane–light petroleum mixture as

colourless small prisms, m.p. 259–260° (lit. 264–265°).[9] (Found: C, 58·9; H, 3·8. Calc. for $C_{19}H_{14}O_9$: C, 59·0; H, 3·6%.)

Hydrolysis with Emulsin

Amritoside (0·02 g) was dissolved in water (5 ml), emulsin solution (50 ml) prepared from sweet almonds[16] added and kept at 37° for 4 days. Ellagic acid started precipitating out after a few hours. After 4 days the solid that came down was centrifuged, washed with water and then with alcohol. In paper chromatography it gave a single pink ring, R_f 0·3 (solvent B, spray: bromophenol blue), m.p. above 360°, was sparingly soluble in water and gave no Molisch test. It was identical with ellagic acid in all respects. The solution was evaporated on a water bath and the syrup on paper chromatogram gave a single ring, R_f 0·58 (solvent E, spray: aniline hydrogen phthalate). It gave a phenylosazone as long yellow needles, identical with the phenylosazone of glucose.

Periodic Acid Oxidation (Quantitative)

The diazomethane methylation product of amritoside (21·27 mg) was dissolved in ethanol (20 ml) and sodium metaperiodate solution (20 ml, 0·01764 M) was added and the mixture (*R*) kept at 35° for 24 hr. An aliquot of this reaction mixture was treated with an excess of standard sodium arsenite solution and potassium iodide solution (added in succession) and the mixture was kept at room temperature (33°) for 20 min. It was then titrated against standardized iodine solution. The titration was repeated three times. Allowing for the blank, the amount of sodium metaperiodate consumed was found to be 4·2 moles per mole of the ellagic acid glycoside methyl ether.

Estimation for Formic Acid

The above reaction mixture (*R*) (20 ml) was titrated potentiometrically against potassium hydroxide solution (0·1004 N). From the volume of alkali consumed the amount of formic acid was found to be 2·0 moles per mole of the glycoside methyl ether. That the presence of free periodate and iodate do not interfere was confirmed by titration of an artificial mixture containing these and formic acid, and also from an exploratory experiment using maltose.

Extraction of Stem Bark: Method (2)

In order to ensure that no flavonoid compound other than leucocyanidin was present in the bark, even in trace quantities, hot ethanol extraction was carried out. Freshly cut inner stem bark (200 g) was extracted with boiling ethanol (0·5 l.) (3 × 8 hr). The combined extract (1·5 l.) was concentrated under reduced pressure (0·2 l.). The solution was decanted from the chlorophyll and wax which had separated out. To this solution (50 ml) conc. sulphuric acid (2 ml) was added in order to make it 7% acid. It was refluxed on a water bath for 2 hr, when the solution became deep red due to the formation of cyanidin. Phlobaphene-like material which separated out on cooling was filtered off. The solution was then continuously extracted with ether for 20 hr. Ether extract showed four definite rings (solvent A), the outermost (R_f 1·0) was visibly yellow and corresponded to carotenoids; the next (R_f 0·90) was pink and corresponded to cyanidin and, on exposing the chromatogram to ammonia, two more deep-yellow rings (R_f 0·35 and 0·56) were visible. On spraying the chromatogram with alcoholic ferric chloride the red cyanidin ring and the two inner rings became blue. Separation of the substances corresponding to these R_f values is described below.

[16] F. G. MANN and B. C. SAUNDERS, *Practical Organic Chemistry*, p. 365, Longmans (1936).

When the ether extract (approx. 30 ml) was kept at room temperature (29) overnight a colourless solid (*G*) crystallized out as long needles. The solution (approx. 10 ml) was decanted and, on keeping, deposited a further crop of the same crystals. The remaining deep-yellow ether solution (2-3 ml) was checked for the presence of flavonoid compounds; it gave only a feeble pink colour with magnesium and hydrochloric acid indicating the presence of a very small amount. This ether solution was completely evaporated and the residue was treated with light petroleum which extracted out all the yellow colour. The petroleum extract on paper chromatogram showed only the yellow ring which travelled with the solvent front; that this was due to the presence of some carotenoid compound was proved by the deep-blue colour the ether solution gave with conc. sulphuric acid. The petroleum-insoluble residue was fairly soluble in boiling alcohol and crystallized out on cooling as long needles, agreeing with (*G*). The solution contained traces of cyanidin. The solid (*G*) was found to be a mixture of ellagic acid and the corresponding hydroxy carboxylic acid and were characterized as already mentioned above.

Extraction of Wood

The wood shavings of the guava tree (100 g) were extracted with ethanol in the cold and the extract worked up as in the case of the bark. From the ethyl acetate solution a very small amount of leucocyanidin could be isolated. This was converted into the anthocyanidin which was identified as cyanidin by mixed paper chromatography and colour tests, λ_{max} at 540 mμ. No other polyphenol could be detected.

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