Phytochemistry, 1965, Vol. 4, pp. 989 to 992. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

POLYPHENOLS OF THE LEAVES OF *PSIDIUM GUAVA*— QUERCETIN, GUAIJAVERIN, LEUCOCYANIDIN AND AMRITOSIDE

T. R. SESHADRI and KRISHNA VASISHTA

Department of Chemistry, University of Delhi, Delhi

(*Received* 29 *October* 1964)

Abstract—From an ethanolic extract of the fresh green leaves was obtained quercetin and its 3-arabinopyranoside, guaijaverin; a high yield of leucocyanidin which gave a single methyl ether; a small amount of ellagic acid, and a high yield of its 4-gentiobioside, identical with amritoside found earlier[1] in the stem bark.

IN AN earlier paper,[1] the polyphenols present in the stem bark of this plant were described. The astringency possessed by the bark was attributed to the high yield of leucocyanidin and of a new water-soluble diglucoside of ellagic acid (amritoside).

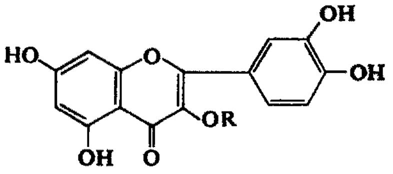
The leaves of this plant have been employed for dyeing and tanning. Previous investigations have shown the presence of wax, resins, sugars, essential oils,[2,3] β-sitosterol[4] and a mixture of four triterpenoid acids.[5] The presence of quercetin and its two isomeric 3-α-arabinosides, avicularin and guaijaverin (I) which have furanose and pyranose forms of the sugar moiety respectively, have also been recorded.[6] As these components do not account for all the properties mentioned above, the fresh green leaves have now been reinvestigated.

The concentrated ethanolic extract of the leaves was fractionated by successive extraction with solvents. Light petrol removed chlorophyll and carotenoids along with triterpenoid acids and other lipid components. Subsequent ether extraction gave quercetin and guaijaverin (I). A minor amount of a glycoside of ellagic acid was present in the moist ether solution and could be separated from quercetin by virtue of its low solubility in dry ether. During the present study, only one arabinoside of quercetin, guaijaverin (I) having the pyranose ring in the sugar moiety was isolated from the leaves; neither the corresponding furanoside, i.e. avicularin, nor any potassium salt of quercetin as reported by Mohammed *et al.*[6] was obtained. The rotation and spectral properties of guaijaverin have now been recorded, as they have not been reported earlier. Ultraviolet spectra in the presence of various reagents are in agreement with its being a 3-arabinoside. Besides m.p., the specific rotation is useful for differentiation between the two arabinosides. Guava leaves seem to be the only source for guaijaverin so far studied.

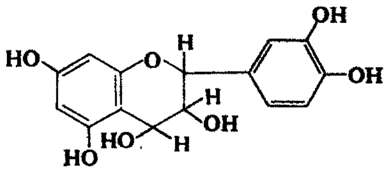
---

[1] T. R. SESHADRI and K. VASISHTA, *Phytochem.* 4, 317 (1965).
[2] G. SOLIMAN and M. K. FARID, *J. Chem. Soc.* 134 (1952).
[3] A. BHATI, *Perfumery Essent. Oil Record* 44, 46 (1953).
[4] H. R. ARTHUR and W. H. HUI, *J. Chem. Soc.* 2782 (1954).
[5] H. R. ARTHUR and W. H. HUI, *J. Chem. Soc.* 1403 (1954).
[6] H. ELKHADME and Y. S. MOHAMMED, *J. Chem. Soc.* 3320 (1958).

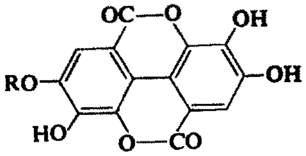
Ethyl acetate extraction gave leucocyanidin (II) which was converted into its acetate, methyl ether and methyl ether acetate and also into cyanidin. It gave only one methyl ether, similar to the soluble methyl ether of leucocyanidin obtained from the stem bark. Leucocyanidin was accompanied by traces of the glycoside of ellagic acid obtained in larger quantities at a later stage.



(I) R = Arabopyranose



(II)



(III) R = Gentiobiose

The remaining aqueous alcoholic mother liquor was found to contain mainly free ellagic acid admixed with traces of its glycoside. The latter being water-soluble could be easily separated from its aglycone. Extraction of the leaves using aqueous alcohol increased the yield of the glycoside though the yields of the other components were lower. The product was identical in colour reactions, m.p., solubility, paper chromatography, u.v. and i.r. spectra with the ellagic acid glycoside (amritoside) (III), isolated from the stem bark and described in a previous paper.[1] In order to be sure of the identity, the position of the sugar moiety as well as the nature of the glycosidic link were examined and gave the same results as before.[1] An observation which was not recorded earlier and which is in agreement with the proposed constitution is the lack of response to Greissmayer's reaction by both the glycoside as well as by the trimethyl ether obtained by methylation and hydrolysis. The result is in accordance with the generalization of Lederer[7] that two free 4,4'-hydroxyls are necessary for the reaction to be positive. The recent report of Srivastava *et al.*[8] that the 3:3'-dimethyl ether of ellagic acid, isolated by them from *Sonneratia apetala*, did not give this reaction may require revision.

It has been reported by Bate-Smith[9] that myricetin and leucodelphinidin are invariably associated with ellagic acid, in the family Myrtaceae which exhibits the most systematic distribution of ellagic and gallic acid derivatives, and that this association holds for almost all the genera and most of the species. But in the guava plant which belongs to the same family the position is different; leucocyanidin is consistently present in all the parts of the plant. Accompanying it in the leaves are found the flavonoids, quercetin and its arabinoside guaijaverin. All the parts contain gallic acid derivatives; a gallotannin is present mainly in the root bark, and in the stem bark and leaves, ellagic acid and its diglucoside are prominent.

---

[7] E. Lederer, *J. Chem. Soc.* 2117 (1949).

[8] S. N. Srivastava, D. S. Bhakuni, V. N. Sharma and K. N. Kaul, *J. Sci. Indian Res.* (*India*), **21B**, 549 (1962).

[9] E. C. Bate-Smith, *Chem. & Ind.* (*London*), *B.I.F. Review*, R-32 (1956).

These results do not support a direct biogenetic relationship between the flavonoids and the gallo- and ellagitannins.

EXPERIMENTAL

R_f values relate to circular paper chromatograms, the irrigating solvents being (A) *m*-cresol saturated with water, (B) phenol–water (upper layer), (C) butanol saturated with ammonia, (D) butanol:acetic acid:water (4:1:5) lower layer and (E) ethanol:water:ammonia (20:4:1).

Extraction of Guava Leaves.

Fresh green leaves (1 kg) were cut and extracted with ethanol in the cold (3 × 5 l.) for 48 hr. The combined extract was concentrated under reduced pressure (500 ml), when most of the chlorophyll and waxy matter separated out. The solution was decanted, diluted with water (250 ml) and continuously extracted with light petrol for 48 hr.; this removed all the remaining waxy and oily matter, chlorophyll, triterpenoids and carotenoids.

Extraction with ether; isolation of flavonoids (*quercetin and guaijaverin*). The remaining deep yellow aqueous alcoholic concentrate was subjected to continuous extraction with ether for 18 hr. A light yellow, crystalline solid separated out from the ether as well as the aqueous solution. Both were worked up separately and crystallized from aqueous ethanol yielding colourless prisms, m.p. 238–240° and 239–240° respectively; the mixed m.p. was undepressed, total yield, 0·35 g. This compound was found to correspond in its properties with guaijaverin described later. The deep yellow ether solution gave a marked bluish tinge with ferric chloride, which was an indication of the presence of a small amount of ellagic acid glycoside as described later. The ether solution was then dried with $MgSO_4$ and evaporated. The residue was extracted with small amounts of dry ether in order to leave behind the small amount of ellagic glycoside. This ether solution gave pure quercetin, m.p. 313°, mixed m.p. with authentic quercetin was undepressed (yield, 0·2 g). The mother liquor was kept saturated with ether for 2 days in the refrigerator when a further quantity of guaijaverin separated out (0·2 g) as colourless flakes.

The guaijaverin sample had all the properties described in the literature[6] (Found: C, 53·3; H, 4·5. Calc. for $C_{20}H_{18}O_{11},H_2O$: C, 53·1; H, 4·5%); $[\alpha]_D^{25}$ −97° (95% ethanol); the rotation recorded for avicularin is $[\alpha]_D^{25}$ −168° (95% ethanol). Ultraviolet spectra: λ_{max} in alcohol 255, 360; with sodium acetate 273, 390; with aluminium chloride 271, 389; with boric acid–sodium acetate 265, 370; with sodium ethoxide 283, 390 mμ. Infrared (KBr): The main peaks were 3450 cm^{-1}, 1645 cm^{-1}, 1600 cm^{-1}, 1550 cm^{-1}, 1490 cm^{-1}, 1440 cm^{-1}, 1350 cm^{-1}, 1285 cm^{-1}, 1225 cm^{-1}, 1190 cm^{-1}, 1110 cm^{-1}, 1080 cm^{-1}, 1050 cm^{-1}, 1000 cm^{-1}, 935 cm^{-1}, 880 cm^{-1}, 812 cm^{-1}, 790 cm^{-1}, 762 cm^{-1}, 690 cm^{-1} and 680 cm^{-1}. When boiled with 7% sulphuric acid for 2 hr at 100° it gave quercetin and arabinose.

Extraction with ethyl acetate (*leucocyanidin*). The remaining aqueous alcoholic extract was continuously extracted with ethyl acetate for 17 hr. The extract (150 ml) was concentrated under reduced pressure to about half its volume and on keeping it in the cold a very small amount (8 mg) of a colourless compound separated out, which was readily soluble in water, gave bluish-green precipitate with ferric chloride and positive Molisch test and was found to be a glycoside of ellagic acid, described later. The decanted ethyl acetate solution (70 ml), which gave an olive-green colour with ferric chloride, was dried ($MgSO_4$) and concentrated under reduced pressure. On adding light petroleum a sticky mass was obtained.

It was taken up with ethyl acetate and by repeated fractional precipitation with light petroleum, leucocyanidin was obtained as colourless prisms. Yield 1·5 g, R_f, 0·31 (solvent: A, spray: dilute hydrochloric acid solution of vanillin). An authentic sample of leucocyanidin under similar conditions gave R_f, 0·32. It gave cyanidin by boiling with 10% ethanolic hydrochloric acid for 2 hr; circular R_f, 0·72 (solvent: B), λ_{max} 545 mμ in 0·1% ethanolic hydrochloric acid.

The leucocyanidin gave an acetate, which crystallized from ethyl acetate–light petroleum mixture as small prisms, m.p. 184–186°, $[\alpha]_D^{32}$ −15° (ethyl acetate) (Found: C, 58·4; H, 5·0. Calc. for $C_{27}H_{26}O_{13}$: C, 58·1; H, 4·7%). Its methyl ether crystallized from methanol as colourless prisms, m.p. 154–156°; $[\alpha]_D^{33\cdot8}$ +14° (ethyl acetate) (After drying for 3 hr. Found: C, 58·5; H, 5·7; Calc. for $C_{19}H_{22}O_7,1\frac{1}{2}H_2O$: C, 58·6; H, 6·4%; after drying for further 4 hr Found: C, 61·2; H, 6·1; Calc. for $C_{19}H_{22}O_7,\frac{1}{2}H_2O$: C, 61·4; H, 6·2%). The acetate of the methyl ether melted at 150–151° (Found: C, 61·7; H, 5·9. Calc. for $C_{23}H_{26}O_9$: C, 61·9; H, 5·9%).

During the extraction of the aqueous alcoholic mother liquor with ethyl acetate a colourless solid separated out from the aqueous layer, as mentioned earlier, which crystallized from alcohol containing a little pyridine as almost colourless long needles, m.p. above 360°, yield 0·15 g. It was identified as ellagic acid by colour tests, mixed chromatography and u.v. spectra.

Ellagic acid diglucoside (*amritoside*). The mother liquor left after removal of free ellagic acid was evaporated completely over potash at room temperature, when a thick brown syrup was obtained. This was extracted repeatedly with small amounts of boiling water:ethanol mixture (1:5). A colourless substance crystallized out as rectangular plates from the extracts on standing. The unextracted residue was dissolved in minimum amount of water and alcohol slowly added to throw out coloured impurities first and finally colourless plates were obtained (total yield, 0·85 g). Final crystallization with water:alcohol mixture (1:5 v/v) yielded big colourless rectangular plates, m.p. 248–250° (decomp.) (Found: C, 50·2; H, 3·7. $C_{26}H_{26}O_{18}$ required: C, 49·8; H, 4·2%). It gave a single ring, R_f 0·32 (solvent C; spray, bromophenol blue) and R_f 0·40 (solvent D; spray, ammonia). In its solubility, colour tests and spectral data it was similar to amritoside.

When hydrolysed with 7% sulphuric acid (2 hr, 100°) it gave ellagic acid and 2 M glucose. Its acetate crystallized from ethyl acetate–light petroleum mixture as colourless plates, m.p. 208–210°, $[\alpha]_D^{25}$ +91·6 (ethyl acetate) (Found: C, 53·0; H, 4·8. $C_{46}H_{46}O_{28}$ required: C, 52·8; H, 4·4%). When methylated with diazomethane and hydrolysed, the glycoside gave 3,3′,4′-tri-*O*-methyl ellagic acid as rectangular prisms, m.p. 290° and mixed m.p. with an authentic sample was undepressed. It gave a single ring with R_f, 0·4 (solvent E) (Found: C, 59·0; H, 3·9. Calc. for $C_{17}H_{12}O_8$: C, 59·3; H, 3·5%). This trimethyl ether gave a monoacetate as colourless small prisms, m.p. 259–260°, undepressed by the sample from stem bark (Found: C, 58·8; H, 3·8. Calc. for $C_{19}H_{14}O_9$: C, 59·0; H, 3·7%). It was unaffected by Taka diastase, but with emulsin yielded ellagic acid and 2 M glucose.