675. *Constituents of the Leaves of* Psidium guaijava, *L. Part II.* Quercetin, Avicularin, and Guaijaverin.*

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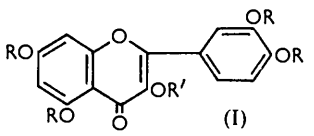
Three antibacterial compounds were isolated from guava leaves: quercetin; avicularin, the 3-L-arabofuranoside of quercetin; and guaijaverin, the 3-α-L-arabopyranoside of quercetin.

In continuation of earlier work (Part I) the active principles of the leaves of *Psidium guaijava* L. have been examined. The alcoholic extract and aqueous decoction showed antibacterial activity,[1] *in vitro* against *Staphylococcus aureus* and *in vivo* in mice infected with *H. Strep.* "Richards". Quercetin (I; R = R′ = H), avicularin, and another quercetin arabinoside (named guaijaverin) were isolated from the ethanolic extract of the defatted leaves. These three compounds inhibit the growth of *Staphylococcus aureus* at a dilution of 0·1 mg./ml., a value similar to that found previously for quercetin.[2] Avicularin, a 3-L-arabinoside of quercetin, was first isolated by Ohta[3] from *Polygonum aviculare* and was later found in *Vaccinium myrtillus*.[4] The structure assigned by Ohta has been confirmed and two new crystalline derivatives of avicularin have been prepared. Complete methylation with dimethyl sulphate and sodium hydroxide, followed by hydrolysis of the methylated glycoside, yielded 5 : 7 : 3′ : 4′-tetramethylquercetin (I; R = Me, R′ = H) and 2 : 3 : 5-tri-*O*-methylarabinose. The latter was converted by oxidation into 2 : 3 : 5-tri-*O*-methylarabonic acid, identified as its crystalline amide. It was concluded that arabinose was present in the furanose form so that avicularin would be the 3-L-arabofuranoside of quercetin.



(I)

Guaijaverin gave on hydrolysis quercetin and L-arabinose, the latter identified as the *N*-benzyl-*N*-phenylhydrazone. It was converted with diazomethane into a tetramethoxy-derivative which on hydrolysis yielded 5 : 7 : 3′ : 4′-tetramethoxyquercetin showing that, like avicularin, guaijaverin is a 3-L-arabinoside of quercetin. Complete methylation followed by hydrolysis yielded 5 : 7 : 3′ : 4′-tetramethylquercetin and 2 : 3 : 4-tri-*O*-methylarabinose, both crystalline, suggesting that the arabinose in guaijaverin is in the pyranose form. This was confirmed by synthesis; acetobromoarabinose was treated with 5 : 7 : 3′ : 4′-tetramethylquercetin and silver carbonate, according to Koenigs and Knorr's method,[5] giving 5 : 7 : 3′ : 4′-tetramethylguaijaverin triacetate, identical with that obtained by acetylation of tetramethylguaijaverin. The Koenigs–Knorr synthesis is known to produce the α-isomer in the case of L-arabinose,[6] so that guaijaverin would be the 3-α-L-arabopyranoside of quercetin.

The ultraviolet absorption maxima of avicularin, guaijaverin, and their acetates and methoxy-derivatives are shown in the Table.

Experimental

Absorption spectra were determined with a Unicam S.P. 500 spectrophotometer. Microanalyses are by A. Bernhardt, Mülheim, W. Germany.

Isolation of Crude Gum.—Powdered dried guava leaves (6·2 kg.) were defatted with light petroleum (b. p. 58—72°), then extracted with cold ethanol (4 × 8 l.). The green ethanol

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[1] Bacteriological tests were made by Professor G. A. H. Buttle, School of Pharmacy, London, and continued by Dr. H. Mazloum, Faculty of Medicine, Alexandria University.

[2] Naghski, Copley, and Couch, *Science*, 1947, **105**, 125.

[3] Ohta, *Z. physiol. Chem.*, 1940, **263**, 221; *Ann. Report Tokyo Coll. Pharmacy*, 1955, 38.

[4] Ice and Wender, *J. Amer. Chem. Soc.*, 1953, **75**, 50.

[5] Koenigs and Knorr, *Sitzb. Bayr. Akad. Wiss.*, 1900, **30**, 103; *Ber.*, 1901, **34**, 957.

[6] Pigman and Goepp, "Chemistry of the Carbohydrates," Academic Press Inc., New York, 1948, p. 103.

extracts were concentrated to 2 l., and hot water (1·5 l.) was added, giving a heavy green precipitate which was filtered off and washed repeatedly with hot water (290 g.). The combined filtrate and washings were concentrated under reduced pressure to about 0·5 l. and dried in a vacuum-desiccator, yielding a brown hygroscopic gum (150 g.).

Ether-extraction of the Crude Gum.—The brown gum (150 g.) was dissolved in water (500 ml.), filtered from a small insoluble residue, and subjected to continuous ether-extraction for 72 hr. The ether soon acquired a yellow tint and after 4 hr. started to deposit in the ether reservoir a crystalline yellow precipitate of crude avicularin, m. p. 195°, which was collected by decantation after being washed with ether (yield 5 g.). The ether-soluble material recovered on evaporation was a yellow gum which crystallised from dilute ethanol, giving quercetin (1·1 g.), m. p. and mixed m. p. 313°. Another yellow crystalline product was deposited in the bottom of the aqueous reservoir and was separated by filtration after being washed with dilute ethanol (3·2 g.). This product melted over a wide range (220—240°) and was found to be a mixture of guaijaverin and a potassium salt of quercetin.

Quercetin.—Apart from the ether-extract quercetin was obtained by boiling the crude gum (14 g.) with acetic acid (30 ml.), filtering the mixture, and adding water (20 ml.). This quercetin (120 mg.), purified by repeated crystallisation from dilute ethanol, also had m. p. and mixed m. p. 313° (Found: C, 59·9; H, 3·4. Calc. for $C_{15}H_{10}O_7$: C, 59·6; H, 3·3%).

Potassium Salt of Quercetin.—The product deposited in the aqueous reservoir of the ether-extractor was washed with dilute ethanol (3·2 g.) and boiled with ethanol (2 × 50 ml.), and the undissolved potassium salt of quercetin was filtered off and crystallised from water (yield, 1·1 g.). The ethanol filtrate, on concentration and addition of warm water, deposited guaijaverin, m. p. 235° (2·1 g.). The potassium salt (588 mg.) in boiling dilute hydrochloric acid precipitated quercetin which, crystallised (401 mg.) from dilute ethanol, had m. p. and mixed m. p. 313° (Found: C, 59·9, 59·9; H, 3·7, 3·6%). The mother-liquor after separation of quercetin was evaporated to dryness on the water-bath and the residue identified as potassium chloride.

Avicularin.—Crude avicularin obtained from the ether-extraction was repeatedly crystallised from dilute ethanol; it formed bright yellow needles, m. p. and mixed m. p. 217° (Found: C, 53·4; H, 4·3. Calc. for $C_{20}H_{18}O_{11},H_2O$: C, 53·1; H, 4·4%). On dehydration avicularin had m. p. 222°.

Hydrolysis. Avicularin (118 mg.) was hydrolysed with 3% sulphuric acid for 1 hr. at 100°; the deposited quercetin (80 mg.), crystallised from dilute ethanol, had m. p. and mixed m. p. 313° (Found: C, 59·9, 59·7; H, 3·4, 3·4. Calc. for $C_{15}H_{10}O_7$: C, 59·6; H, 3·3%). The hydrolysate after separation of quercetin was neutralised with barium carbonate, filtered, and passed through a cation-exchange resin. The colourless solution was evaporated to dryness, giving L-arabinose, m. p. 157°, $[\alpha]_D$ +98·5° (*c* 0·64 in H_2O). One-dimensional paper chromatograms of this product were run on Whatman No. 1 filter paper and developed with the upper layer of butan-1-ol–ethanol–water–ammonia (40 : 10 : 49 : 1). Spraying with 10% aqueous ammonium molybdate [7] and heating at 100° showed one spot, R_F 0·19; controls showed an identical value for L-arabinose. Another portion of arabinose from the hydrolysis was converted into the *N*-benzyl-*N*-phenylhydrazone, m. p. 173° alone or mixed with L-arabinose *N*-benzyl-*N*-phenylhydrazone.

Avicularin hepta-acetate. A solution of avicularin in dry pyridine was acetylated with acetic anhydride in the usual manner. The amorphous acetate obtained crystallised in one week and was purified by crystallisation from chloroform–light petroleum (b. p. 100—120°), forming colourless needles, m. p. 187°, $[\alpha]_D$ −136° (*c* 1·22 in $CHCl_3$). *Avicularin hepta-acetate* is soluble in chloroform or hot ethanol, and insoluble in light petroleum (Found: C, 56·1, 55·9; H, 4·5, 4·5. $C_{34}H_{32}O_{18}$ requires C, 56·0; H, 4·4%).

Tetra-O-*methylavicularin.* Avicularin (920 mg.), dissolved in methanol (50 ml.), was treated with 2% diazomethane in ether (150 ml.) and left to evaporate slowly at room temperature. After two days, the crystals that separated were filtered off and crystallised from chloroform–light petroleum (b. p. 100—120°) in colourless needles, m. p. 225°. *Tetra*-O-*methylavicularin* is soluble in chloroform or hot ethanol and insoluble in light petroleum (Found: C, 58·4; H, 5·2. $C_{24}H_{26}O_{11}$ requires C, 58·8; H, 5·3%).

Hydrolysis of tetramethylavicularin. A solution of tetra-*O*-methylavicularin (600 mg.) in hot ethanol (20 ml.) was refluxed with 3% sulphuric acid (20 ml.) on the water-bath for 2 hr.

[7] Spraying agent for reducing sugars: El Khadem and Hanessian, unpublished work.

5 : 7 : 3′ : 4′-Tetramethoxyquercetin obtained was crystallised from ethanol (370 mg.), then having m. p. and mixed m. p. 194° (Found: C, 64·1, 64·0; H, 5·1, 5·2. Calc. for $C_{19}H_{18}O_7$: C, 63·7; H, 5·0%).

Complete methylation and hydrolysis of avicularin. A solution of avicularin hepta-acetate (1·1 g.) in acetone (200 ml.) was stirred with 35% aqueous sodium hydroxide (30 ml.). Dimethyl sulphate (10 ml.) was added during 2 hr. and the mixture heated to 80° to distil off acetone. The brown solution was then extracted with chloroform and the latter washed with water, dried, and evaporated, giving a brown gum of completely methylated avicularin. This was hydrolysed with 3% sulphuric acid for 3 hr. at 100°, giving 5 : 7 : 3′ : 4′-tetramethylquercetin, m. p. and mixed m. p. 194°.

The hydrolysate, after separation of tetramethylquercetin, was neutralised with barium carbonate, filtered, passed through a column of cation-exchange resin, and evaporated, giving a yellowish gum of methylated arabinose. This was oxidised with bromine water for 12 hr. at room temperature and excess of bromine removed by bubbling air through the solution. Hydrogen bromide was removed with silver carbonate and the filtrate passed through a cation-exchange resin. The acid obtained was distilled at 130°/6 mm., giving a colourless amorphous lactone, which was treated with ethanolic ammonia for 24 hr. at 0°. The amide obtained on evaporation crystallised from acetone, then having m. p. 140°, not depressed on admixture with 2 : 3 : 5-tri-*O*-methylarabonamide.

Guaijaverin.—Crude *guaijaverin* (2·1 g.) obtained after separation of the potassium salt of quercetin was purified by crystallisation from dilute ethanol in bright yellow needles, m. p. 239°, and, on dehydration, m. p. 256°. It is sparingly soluble in ethanol, methanol, and acetone (Found: C, 53·4; H, 4·7. $C_{20}H_{18}O_{11},H_2O$ requires C, 53·1; H, 4·4%).

Hydrolysis of guaijaverin. Guaijaverin (104 mg.) was hydrolysed with 3% sulphuric acid (20 ml.) for 1 hr. at 100° and the deposited quercetin (69 mg.) crystallised from dilute ethanol (m. p. and mixed m. p. 313°) (Found: C, 59·4, 59·6; H, 3·6, 3·6%).

The hydrolysate after separation of quercetin was neutralised with barium carbonate, filtered, and passed through a cation-exchange resin. The colourless solution was evaporated, giving L-arabinose, m. p. 157°, $[\alpha]_D$ +97·2° (*c* 0·55 in H_2O), identified as in the previous case.

Guaijaverin hepta-acetate. A solution of guaijaverin in dry pyridine was treated with acetic anhydride, and the *hepta-acetate* crystallised from chloroform–light petroleum (b. p. 100—120°) in colourless needles, m. p. 226°, $[\alpha]_D$ −102° (*c* 0·54 in $CHCl_3$) (Found: C, 56·2, 56·2; H, 4·6, 4·6. $C_{34}H_{32}O_{18}$ requires C, 56·0; H, 4·4%).

Tetra-O-methylguaijaverin. Guaijaverin (914 mg.) in methanol (50 ml.) was treated with 2% diazomethane in ether (150 ml.), and the mixture left to evaporate slowly at room temperature. After 2 days the crystals that separated were filtered off and crystallised from chloroform–light petroleum (b. p. 100—120°) in nearly colourless needles, m. p. 247°. *Tetra-O-methylguaijaverin* is soluble in chloroform, difficultly soluble in hot ethanol or methanol, and insoluble in light petroleum (Found: C, 59·2, 58·9; H, 5·4, 5·3; OMe, 24·8. $C_{24}H_{26}O_{11}$ requires C, 58·8; H, 5·3; OMe, 25·3%).

Hydrolysis of tetramethylguaijaverin. A solution of tetramethylguaijaverin (650 mg.) in hot ethanol (20 ml.) was refluxed with 3% sulphuric acid (20 ml.) on the water-bath for 2 hr. 5 : 7 : 3′ : 4′-Tetramethylquercetin obtained was crystallised from ethanol (yield, 420 mg.; m. p. and mixed m. p. 194°) (Found: C, 63·9; H, 5·1. Calc. for $C_{19}H_{18}O_7$: C, 63·7; H, 5·0%).

Tetra-O-methylguaijaverin triacetate. Tetramethylguaijaverin (60 mg.) in dry pyridine (2 ml.) was treated with acetic anhydride, and the *acetate* obtained crystallised from ether in needles, m. p. 159—160°, soluble in chloroform or hot ethanol, and insoluble in light petroleum (Found: C, 58·3, 58·5; H, 5·4, 5·3. $C_{30}H_{32}O_{14}$ requires C, 58·4; H, 5·2%).

Complete methylation and hydrolysis of guaijaverin. A solution of guaijaverin hepta-acetate (1·1 g.) in acetone (200 ml.) was treated with 35% aqueous sodium hydroxide (30 ml.) and dimethyl sulphate (10 ml.) as in the preceding case. The methylated guaijaverin was hydrolysed with 3% sulphuric acid (20 ml.) at 100° for 3 hr., giving 5 : 7 : 3′ : 4′-tetramethylquercetin, m. p. and mixed m. p. 194°; the liquor gave, as above, 2 : 3 : 4-tri-*O*-methylarabinose, m. p. and mixed m. p. 82°.

Synthesis of Tetra-O-methylguaijaverin Triacetate.—A solution of 5 : 7 : 3′ : 4′-tetramethylquercetin (30 mg.) and acetobromoarabinose (650 mg.) in dry benzene (25 ml.) was refluxed with silver carbonate (2 g.) for 2 hr., then filtered from the suspended silver salts. The clear solution was evaporated to dryness under reduced pressure and extracted with boiling ether (2 × 50 ml.);

and the ether extracts were filtered and concentrated to 5 ml. Colourless needles of 5 : 7 : 3′ : 4′-tetramethylguaijaverin triacetate separated and were crystallised from ether; they had m. p. and mixed m. p. 159—160°.

Absorption spectra.

Substance	Solvent	$\lambda_{max.}$ (mμ)	log ε	$\lambda_{min.}$ (mμ)	log ε
Avicularin	EtOH	260	4·32	235	4·07
		360	4·24	285	3·85
Avicularin hepta-acetate	,,	254	4·38	240	4·34
		307	4·33	280	4·08
Tetra-*O*-methylavicularin	$CHCl_3$	250	4·29	280	3·92
		335	4·24		
Guaijaverin	EtOH	260	4·45	235	4·19
		360	4·38	285	3·96
Guaijaverin hepta-acetate	,,	257	4·40	243	4·34
		311	4·38	280	4·15
Tetra-*O*-methylguaijaverin	$CHCl_3$	255	4·42	280	3·81
		345	4·34		

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