28. *Constituents of the Leaves of* Psidium guaijava, *L. Part I. Psidiolic Acid.*

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Guava leaves contain a wax, a phytosterol, and a new triterpene, psidiolic acid. It is obtainable from the alcoholic extract of the defatted leaves, and is shown to have the formula $C_{30}H_{48}O_4$ comprising two hydroxyl groups and a tertiary carboxyl group.

Psidium guaijava, L. (Myrtaceae), an arborescent shrub or small tree, which is noted for its edible fruit, is widely cultivated in Egypt. On account of their nutritive value, guava fruits have been investigated by several workers (Miller and Robbins, *Hawaii Agric. Exp. Stat. Bull.*, 1934—35, 25; Goldberg and Levy, *Nature*, 1941, **148**, 286; *J. S. Afr. Chem. Inst.*, 1942, **25**, 3; Webber, *Proc. Amer. Soc. Hort. Sci.*, 1944, **45**, 87; Quinones, Guerrant, and Dutcher, *Food Res.*, 1944, **9**, 415; Hartzler, *J. Nutrition*, 1945, **30**, 355) and shown to be rich in vitamin C. Similarly, the seed oil was examined by Kinzo, Kafuku, Hata, and Massaichi (*J. Chem. Soc., Japan*, 1934, **55**, 373), and by Varma, Godbole, and Srivatava (*Fettchem.*, 1936, **43**, 8) and its constants were determined. However, guava leaves appear to have escaped systematic investigation and the present knowledge regarding the occurrence of an essential oil is derived from Schimmel's Semi-Annual Rept., 1910, p. 123, and Klein ("Handbuch Der Pflanzenanalyse," J. Springer, 1932, Vol. II, p. 351). Recently, Schroeder (*Stud. Inst. Divi Thomae*, 1946—47, **5**, 1) studied the inhibitory action of the crude extracts of guava leaves on *Entamœba histolytica*.

Since the decoction of guava leaves is commonly used by Egyptians to alleviate cough and pulmonary disorders, we have started a systematic search for an active principle. So far, we have confirmed the presence of about 0·2% of a lemon-yellow oil, but contrary to Klein (*loc. cit.*) we were unable to prove the presence of eugenol in it. Yet the oil showed a tendency to resinify and, after ageing, its alcoholic solution gave a violet-red colour with ferric chloride.

Together with the essential oil, the petroleum extract of the leaves yielded a dark green oil which deposited on cooling an oxygen-containing wax, m. p. 82°, whereas its unsaponifiable fraction provided a crystalline phytosterol, m. p. 135—136°, the properties of which will be communicated later.

The alcoholic extract of the defatted leaves which contains an ether-soluble green resin, tannins, sugars, and an ether-insoluble brown resin proved to be a rich source for a microcrystalline substance, m. p. 252—254°. This was isolated from the ether-soluble resin by treatment with acetone and repeated crystallisation from alcohol.

Analysis indicated that this substance has the formula $C_{30}H_{48}O_4$ and contains a free carboxyl group (titration with alkali). The presence of two hydroxyl groups (primary or secondary) was indicated by the formation of a diacetate, dipropionate, and dibenzoate. Further, the acid was converted into its methyl ester by methyl sulphate or diazomethane. The ester which resisted hydrolysis by alkali was similarly converted into its dipropionate and dibenzoate. The acid gave a pale-yellow colour with tetranitromethane and the usual colour reactions which characterise triterpene acids. It appears that this compound is a new triterpene, now named psidiolic acid.

Unlike hederagenin, methyl psidiolate did not give an *iso*propylidene derivative on treatment with hydrochloric acid in presence of dry acetone (cf. Jacobs, *J. Biol. Chem.*, 1925, **63**, 621) and, in contrast to the isomeric triterpene acids, the presence of a sterically protected double bond in psidiolic acid could not be detected by means of perbenzoic acid. Attempts to prepare a lactonic isomer, the formation of which necessitates the presence of a double bond and a carboxyl group in suitable positions, by the action of hydrogen bromide in acetic acid led to resinification. The action of bromine on the acid or its methyl ester gave traces of the unchanged material as sole definite product. Further experiments are in progress.

Experimental

M. p.s are uncorrected. Microanalyses were by Drs. Weiler and Strauss, Oxford, and the analytical specimens were dehydrated at 110° over phosphoric oxide in a high vacuum.

Extraction with Light Petroleum.—The powdered leaves of *Psidium guaijava* (4 kg.), collected in summer and autumn, were extracted three times with light petroleum (b. p. 60—80°). The combined extracts yielded on concentration a dark greenish oily residue (200 g.) (cf. Abderhalden, " Biochemisches Handlexicon," J. Springer, 1911, Vol. III, p. 138) which deposited crystalline plates on cooling in the ice-chest. The deposit was freed from most of the oil and pigments by treatment with cold methanol, and purified by crystallisation from acetone and recrystallisation from benzene–light petroleum. The crystalline prisms (8 g.), m. p. 81—82°, thus obtained were recovered unchanged after being heated with acetic anhydride–pyridine, and were almost unaffected by refluxing 10% methanolic potassium hydroxide during 10 hours [Found : C, 80·4, 80·6; H, 13·6, 13·8%; *M* (Rast), 391, 413].

After separation of the wax, the greenish oil was freed from the last traces of solvent and then subjected to distillation with steam. The distillate contained a mobile lemon-yellow essential oil (0·2%) which gave a negative ferric chloride reaction. After being kept for several months, the oil became viscous and its alcoholic solution gave a violet-red colour with ferric chloride. Another sample of the essential oil was also prepared by distillation of the fresh leaves with steam, and behaved similarly.

The fatty oil was again recovered and hydrolysed with alcoholic potassium hydroxide, and the unsaponifiable fraction extracted with ether. After evaporation of ether, the orange viscous residue was treated with cold methanol, and the semi-crystalline fraction separated. The solid was purified by washing it with cold light petroleum (b. p. 40—60°), and after several crystallisations from alcohol and acetone was obtained in glistening plates, m. p. 135—136°, which yielded an acetate, m. p. 122°, and gave the colour reactions of sterols.

Extraction with Alcohol.—The defatted leaves (4 kg.) were refluxed with alcohol for 10 hours and filtered hot. This process was repeated three times, and the combined filtrates were evaporated almost to dryness under reduced pressure. The residue which contained a green resin, tannins, sugars, and a brown resin was extracted with boiling ether which dissolved only the green resin.

Psidiolic Acid.—The green resin recovered from the above-mentioned ethereal solution was treated with about 250 ml. of cold acetone, and the insoluble portion crystallised (charcoal) twice from alcohol. The discoloured product (40 g.) was further purified by washing it with acetone and ether, and finally crystallised from alcohol in spheroidal crystals, m. p. 252—254°, $[\alpha]_D^{19}$ +39·9° (*c*, 7·66 in pyridine) [Found : C, 76·5, 76·5, 75·9; H, 10·0, 10·3, 10·4; CO_2H (by titration), 9·3, 9·5, 9·5%; *M* (Rast), 450, 455. $C_{29}H_{47}O_2{\cdot}CO_2H$ requires C, 76·2; H, 10·2; CO_2H, 9·5%; *M*, 472·4]. Pure *psidiolic acid* is insoluble in light petroleum, sparingly soluble in ether, acetone, chloroform, benzene, or ethyl acetate, and is freely soluble in pyridine. It gave a yellowish colour with tetranitromethane, a reddish-violet colour with Liebermann–Burchard reagent (*Ber.*, 1885, **18**, 1803), a deep red colour with Salkowski's reagent (*Z. physiol. Chem.*, 1908, **57**, 523), and an orange ⟶ reddish ⟶ violet ⟶ brown colour with Tschügajeff's reagent (*Chem. Ztg.*, 1900, **24**, 542).

O-*Acyl Derivatives of Psidiolic Acid.*—A solution of the acid (2 g.) in pyridine (15 ml.) and acetic anhydride (15 ml.) was heated on the steam-bath for 3 hours. The product, isolated in the usual manner, crystallised from dilute alcohol in plates and recrystallised from light petroleum (b. p. 80—90°) in hexagonal prisms, m. p. 198—200° (Found : C, 73·0, 73·2; H, 9·5, 9·3. $C_{34}H_{52}O_6$ requires C, 73·3; H, 9·4%). Hydrolysis of the *diacetate* with alcoholic potassium hydroxide gave psidiolic acid, m. p. and mixed m. p. 252—254°. The *dipropionate*, similarly prepared, crystallised from dilute alcohol in needles and recrystallised from light petroleum (b. p. 80—90°) in prisms, m. p. 225—226° (Found : C, 73·8; H, 9·6. $C_{36}H_{56}O_6$ requires C, 73·9; H, 9·7%). The *dibenzoate* was prepared by heating a solution of psidiolic acid (2 g.) in pyridine (20 ml.) with benzoyl chloride (2 ml.) for 4 hours at 50°; after being kept at room temperature for 24 hours, the mixture was poured into water. The product was thoroughly washed and the resinous impurities removed by digestion with about 30 ml. of methanol. The dibenzoate crystallised from benzene–alcohol in glistening prisms, m. p. 248—250° [Found : C, 78·0, 78·2, 78·3, 78·5; H, 7·6, 7·7, 7·8, 8·0%; *M* (by titration), 666·2, 663·4. $C_{44}H_{56}O_6$ requires C, 77·6; H, 8·3%; *M*, 680·45]. Better analytical figures could not be obtained from the highly purified specimens. $[\alpha]_D^{19}$ was +0·06° (*c*, 2·76 in benzene).

Methyl Psidiolate.—Methyl sulphate (15 ml.) was gradually added, with shaking, to a

solution of psidiolic acid (5 g.) in alcohol (60 ml.) and 10% sodium hydroxide (60 ml.). After addition of more alkali and methyl sulphate, the mixture was kept at room temperature for 2 hours and the crystalline *ester* collected. It crystallised from dilute methanol and recrystallised from benzene–light petroleum (b. p. 60—80°) in glistening needles, m. p. 213—215°, after sintering at 150°, $[\alpha]_D^{19}$ +52·1° (*c*, 5·26 in ethanol) (Found: C, 76·3, 76·4; H, 10·3, 10·4; OMe, 6·7. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4; OMe, 6·4%). The ester was recovered unchanged when its solution in dry acetone was treated with a few drops of concentrated hydrochloric acid, and it was not hydrolysed when heated with 5% alcoholic potassium hydroxide for 5 hours.

O-*Acyl Derivatives of Methyl Psidiolate.*—Methyl psidiolate reacted quite readily with acetic anhydride in presence of pyridine or fused sodium acetate, but the acetylation product was freely soluble in most of the organic solvents and did not crystallise from dilute solvents. The *dipropionate* was prepared when a solution of the ester (2 g.) in pyridine (15 ml.) and propionic anhydride (15 ml.) was kept at room temperature for 3 days. The product was isolated in the usual manner and crystallised from dilute alcohol in glistening prisms, m. p. 150° (Found: 74·6; H, 10·0; OMe, 5·9. $C_{37}H_{58}O_6$ requires C, 74·2; H, 9·8; OMe, 5·2%). The *dibenzoate* was prepared by heating a solution of the ester (1 g.) in pyridine (10 ml.) with benzoyl chloride (1·5 ml.) for 8 hours at 40°; after being kept at room temperature for 24 hours, the mixture was poured into water. The resinous product was washed free from pyridine and digested with methanol. The insoluble portion crystallised from benzene–methanol in prisms, m. p. 210°, $[\alpha]_D^{19}$ 0° (*c*, 2·89 in benzene) (Found: C, 78·0, 77·6; H, 8·5, 8·3. $C_{45}H_{58}O_6$ requires C, 77·8; H, 8·4%). Both the dipropionate and the dibenzoate gave the pure ester on hydrolysis with alcoholic potassium hydroxide.

Reactions for Unsaturation.—25-Ml. portions of 0·047N-solution of perbenzoic acid in chloroform were added to 0·2010, 0·1692, and 0·1803-g. portions of the dipropionate of methyl psidiolate, and the three solutions were kept at 0° for 7 days. Afterwards, the reagent which remained almost unaffected was determined in the usual manner and the ester recovered unchanged.

A solution of methyl psidiolate (2 g.) in chloroform (20 ml.) was mixed with 10 ml. of 3% bromine solution in chloroform, and after 2 hours the solvent was evaporated at room temperature. The brownish resinous residue yielded traces of the unchanged ester after crystallisation from benzene–light petroleum.

A solution of psidiolic acid (2 g.) in acetic acid (200 ml.) and water (10 ml.) was mixed with 25 ml. of 3% bromine solution in acetic acid, and after 3 hours the mixture was stirred into water and the yellowish gelatinous product collected. After two crystallisations from methanol followed by washing with acetone, it was obtained as a whitish product (0·4 g.) which recrystallised from alcohol in spheroidal crystals, m. p. 252—254°, not depressed by psidiolic acid, and gave methyl psidiolate with diazomethane.

A solution of diacetyl psidiolic acid (0·6 g.) in acetic acid (10 ml.), saturated with hydrogen bromide, was kept at room temperature for 3 days and then poured into water. The resinous product gave on crystallisation from dilute alcohol and recrystallisation from light petroleum (b. p. 80—90°) traces of the starting material.

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