Triterpene Acids from the Leaves of Psidium guaijava, *L.*

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" Psidiolic acid," isolated from the leaves of *Psidium guaijava*, L., and previously reported as a new triterpene acid, has been shown to be a mixture containing ursolic acid, oleanolic acid, crategolic acid, and a new triterpene acid which we have named guaijavolic acid. Guaijavolic acid, $C_{30}H_{48}O_4$, contains a tertiary carboxyl group and two hydroxyl groups.

Soliman and Farid (*J.*, 1952, 134) reported that they had isolated from the leaves of *Psidium guaijava*, L. (Myrtaceae) a new triterpene acid which they called " psidiolic acid," and we showed (*Chem. and Ind.*, 1952, 693) that the leaves of the local species of *Psidium guaijava* also contained " psidiolic acid." Our material, which separated from organic solvents in spheroidal aggregates and gave a reddish-violet colour in the Liebermann–Burchardt test, had m. p. 254—256°; and the m. p.s of its derivatives corresponded with those stated by Soliman and Farid. Because of the manner in which " psidiolic acid " separated from organic solvents, we believed that it was a mixture.

A sample of " psidiolic acid " was chromatographed on alumina. Development for 80 hr. with acetone followed by elution with ethyl acetate, gave 65 fractions, the first 23 of which consisted almost entirely of ursolic acid. The more soluble oleanolic acid was obtained in very low yield from the filtrates of the fractions containing ursolic acid.

Fractions 26—65 contained a triterpenoid substance (*A*) which separated from aqueous ethanol in clusters of short needles. Microscopic examination of (*A*) after it had separated from ethanol or methanol showed that it was obtainable in the form of " threads " (or " rods "), " arrow heads," or " spheres " thus giving evidence of a metastable mesomorphic state (J. Alexander, " Colloid Chemistry," Vol. 1, Chemical Catalog Co. Inc., New York) 1926). While " psidiolic acid " gives a reddish-violet colour in the Liebermann–Burchardt test, and ursolic acid and oleanolic acid a red ⟶ violet ⟶ blue ⟶ green colour, (*A*, gave a red ⟶ orange-red colour.

Because (*A*) could be obtained crystalline only with difficulty, a mixture was again suspected. A sample of (*A*) was chromatographed on alumina, but no change in properties of any of the fractions was observed. It sublimed unchanged in a high vacuum at 200°; a sample was acetylated and the product chromatographed on alumina by two different procedures, one with petroleum and chloroform, and the other with acetone and ethyl acetate; but neither procedure effected a separation.

However, fractional recrystallisation of the acetate of (*A*) from light petroleum (b. p. 40—60°), a triangular scheme being used, gave after 5 operations the least soluble fraction as fine needles, the m. p. of which could not be raised by further recrystallisation or by chromatography. Hydrolysis of this acetate yielded a new crystalline acid (*B*), $C_{30}H_{48}O_4$, which we have called guaijavolic acid; that it contains a tertiary carboxyl group was shown by titration with alkali and by the preparation of a methyl ester which resists hydrolysis with ethanolic alkali. The presence of two hydroxyl groups was shown by the formation of the diacetate and the methyl ester dipropionate. The acid gives a yellow colour with tetranitromethane and the usual colour reactions characteristic of triterpenoid compounds.

The fraction of the acetate of (*A*) which was more soluble in light petroleum than guaijavolic acid diacetate could not be obtained pure from a repetition of the fractional recrystallisation. When hydrolysed it yielded an acid which melted over a range at about 270°. The methyl ester of this acid, however, gave on fractional recrystallisation from light petroleum (b. p. 40—60°) a product (*C*) which had m. p. 221—223°, alone or on admixture with authentic methyl crategolate. The authentic methyl crategolate was obtained from the leaves of *Crataegus oxyacantha*, L. (Rosaceae) and purified by Tschesche, Heesch, and Fugmann by the method described by them (*Chem. Ber.*, 1953, **86**, 626). The identity of (*C*) with methyl crategolate was finally established by reference to their infra-red absorption spectra.

Tschesche and Fugmann (*ibid.*, 1951, **84**, 810) isolated from the leaves of *Crataegus oxyacantha* an intimate mixture of triterpenoid acids, which they called *cis*- and *trans*-crategolic acids; they considered that crategolic acid was 2 : 19-dihydroxyurs-13(18)-ene-17-carboxylic acid. However, Bersin and Muller (*Helv. Chim. Acta*, 1951, **34**, 1868) showed that the leaves of this plant contained ursolic acid, of which the former workers made no mention. In a second paper (*ibid.*, 1952, **35**, 1891), Bersin and Muller showed that oleanolic acid was also present.

It was shown by Tschesche, Heesch, and Fugmann (*loc. cit.*) that Tschesche and Fugmann's "crategolic acid" contained 60—65% of ursolic acid, since methyl ursolate could be chromatographically removed from the methyl esters of the triterpenoid mixture; the residue gave, on repeated recrystallisation, methyl crategolate identical (m. p., mixed m. p., and infra-red spectrum) with (*C*) obtained by fractional recrystallisation of the methyl esters of the triterpenoid mixture from the leaves of *Psidium guaijava* after removal of ursolic, oleanolic, and guaijavolic acids.

The molecular formula and structure of crategolic acid need elucidation since Tschesche and Fugmann's work (*loc. cit.*) was carried out on material containing a large proportion of ursolic acid. Analysis of purified samples of methyl crategolate from *Crataegus oxyacantha* and *Psidium guaijava* suggest that crategolic acid is a monocarboxylic acid of the formula $C_{30}H_{48}O_4$, as originally suggested by Tschesche and Fugmann, and is possibly isomeric with guiajavolic acid.

There is a similarity in the triterpenoid constituents of both plants since they contain in common, ursolic acid, oleanolic acid, and crategolic acid. It is possible that the leaves of *Crataegus oxyacantha* also contain guaijavolic acid, since Bersin and Muller have shown that the triterpenoid mixture from that plant contains 30·2% of ursolic acid, 2·1% of oleanolic acid, and 40·0% of a mixture not separated. Whilst the yield of the triterpenoid mixture from *Crataegus oxyacantha* is 25%, that from *Psidium guaijava* is 0·7%. We have shown that the mixture from the latter contains 33% of ursolic acid, 1·5% of oleanolic acid, 12% of guaijavolic acid, and 10% of crategolic acid.

Guaijavolic acid diacetate and methyl guaijavolate, after recrystallisation to constant m. p., showed no change in properties when chromatographed. Samples of guaijavolic acid obtained by the hydrolysis of the diacetate before and after chromatography of the diacetate were identical.

EXPERIMENTAL

M. p.s are corrected. Some analyses are by Dr. Zimmermann, Melbourne. Mixed m. p.s with authentic specimens were determined with ursolic acid and oleanolic acid and their derivatives. No depressions were observed. The alumina used for chromatography was B.D.H., analysis grade. Spectra were measured on a Grubb-Parsons S3A spectrometer equipped with a sodium chloride prism; solutions were in carbon disulphide.

Dried powdered leaves (3 kg.) of *Psidium guaijava* were extracted for 60 hr. with petroleum (b. p. 60—80°) and then with cold ether (3 × 10 l.). The ethereal extract was shaken twice with an equal volume of sodium hydroxide solution (1%). On acidification, the brown alkaline extract yielded a green precipitate (45 g.) which after six crystallisations from ethanol (charcoal) yielded white spheroidal aggregates (20 g.), m. p. 254—256°. Attempts to crystallise this mixture from polar and non-polar organic solvents failed. The mixture (10 g.) was dissolved in acetone and chromatographed on alumina (1 kg.); development for 80 hr. with acetone was followed by elution with ethyl acetate. Each fraction (400 ml.) was evaporated to dryness, and the residues were dissolved in ethanol and left to crystallise.

Ursolic Acid.—Fractions 1—23 deposited needles (3 g.) of ursolic acid which after two recrystallisations had m. p. 290—291° (vac.), $[\alpha]_D^{21}$ +67° (*c*, 1·07 in 1 : 1-methanol–chloroform) (Liebermann–Burchardt test: a red ⟶ violet ⟶ blue ⟶ green) (Found: C, 78·7; H, 10·5. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%). It formed ursolic acid acetate, m. p. 293—295° (Found: C, 77·0; H, 9·8. Calc. for $C_{32}H_{50}O_4$: C, 77·1; H, 10·1%), methyl ursolate, m. p. 170° (Found: C, 79·5; H, 10·9. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%), and methyl ursolate acetate, m. p. 246—247° (Found: C, 77·3; H, 10·2. Calc. for $C_{33}H_{52}O_4$: C, 77·3; H, 10·2%). Hydrolysis of ursolic acid acetate (by ethanolic potassium hydroxide) and of methyl ursolate (by potassium hydroxide in ethylene glycol) yielded ursolic acid, m. p. 290—291° (vac.).

Oleanolic Acid.—Five crops of ursolic acid were removed from fractions 1—23. The sixth crop, which was amorphous and had m. p. higher than ursolic acid, yielded after two crystallisations from ethanol, oleanolic acid (0·15 g.) as needles, m. p. 310—312° (vac.) (Liebermann–Burchardt test: red ⟶ violet ⟶ blue ⟶ green). It formed methyl oleanolate, m. p. 201° (Found: C, 79·1; H, 10·6; OMe, 6·4. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7; OMe, 6·6%), and methyl oleanolate acetate, m. p. 219—221°.

Guaijavolic Acid.—Fractions 27—65 [substance (*A*)] (5 g.), obtained by chromatography, had identical properties; fractions 24—26 were mixtures of ursolic, oleanolic, crategolic and guaijavolic acids. Substance (*A*) (Liebermann–Burchardt test: red ⟶ orange-red ⟶ brown) crystallised from aqueous ethanol, from which it separated in clusters of short needles, but did not crystallise from other solvents. Despite repeated recrystallisation and sublimation, m. p.s were variable, ranging from 250° to 270° in open tubes. It had m. p. 290° (vac.) after softening at 255—260°, before or after re-chromatography on alumina.

The acetate of (*A*), after being boiled for an hour in 70% aqueous ethanol in order to decompose mixed anhydrides, did not give a sharp m. p., nor could the m. p. be improved by two different chromatographic procedures. It (5 g.) was fractionally recrystallised from light petroleum (b. p. 40—60°), a triangular scheme being used. The least soluble fraction (1·2 g.) was obtained as fine needles, m. p. 242—244°, not raised by recrystallisation or by chromatography. This was *guaijavolic acid diacetate*, $[\alpha]_D^{32}$ +20·7° (*c*, 1·11 in $CHCl_3$) (Found: C, 73·0; H, 9·0; Ac, 15·4. $C_{34}H_{52}O_6$ requires C, 73·3; H, 9·4; 2Ac, 15·5%), which was hydrolysed by ethanolic potassium hydroxide to *guaijavolic acid* (*B*), m. p. 306—308° (vac.) (from aqueous ethanol), $[\alpha]_D^{32}$ +34·4° (*c*, 0·62 in C_5H_5N) [Found: C, 75·8; H, 10·2%; equiv., 454 (by titration); *M*, 487. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·2%; equiv., 472 (for one carboxyl group); *M*, 472]. It gave a pale yellow colour with tetranitromethane and a red ⟶ orange-red ⟶ brown colour in the Liebermann–Burchardt test. Treatment of guaijavolic acid with ethereal diazomethane gave *methyl guaijavolate*, which crystallised from light petroleum in needles, m. p. 210—211°, $[\alpha]_D^{17}$ +55° (*c*, 0·25 in $CHCl_3$) (Found: C, 76·6; H, 10·2; OMe, 6·3. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4; OMe, 6·4%). *Methyl guaijavolate dipropionate* was prepared by dissolving the methyl ester (0·6 g.) in a mixture of pyridine (4 ml.) and propionic anhydride (4 ml.). The mixture was left for 3 days at room temperature and then poured into water. The product crystallised from ethanol (charcoal) in prisms, m. p. 154—155° (Found: C, 74·5; H, 9·8; OMe, 5·2. $C_{37}H_{58}O_6$ requires C, 74·2; H, 9·8; OMe, 5·2%).

Methyl Crategolate.—All fractions of the more soluble acetylated (*A*) had a m. p. range of several degrees, and a repetition of the fractional crystallisations by processes similar to that which yielded guaijavolic acid diacetate produced nothing of constant m. p. This material (1·9 g.) was hydrolysed and the free acids were converted by means of diazomethane into the methyl esters which were fractionally recrystallised from light petroleum (b. p. 40—60°). The least soluble fraction (1·0 g.) was obtained as fine needles, m. p. 221—223° alone or in admixture with a sample of purified methyl crategolate obtained from Professor Tschesche (Found : C, 76·8; H, 10·4; OMe, 6·3. Calc. for $C_{31}H_{50}O_4$: C, 76·5; H, 10·4; OMe, 6·4%). Both samples of methyl crategolate gave identical infra-red spectra in the range 900—1350 cm.$^{-1}$.

The more soluble methyl esters had a m. p. range, as had the more soluble acetates. These fractions were considered to be mixtures of the methyl esters and the acetates of guaijavolic acid and crategolic acid.

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