Constituents of the Leaves of *Ficus carica*, L. Part II.[1] Isolation of a ψ-Taraxasteryl Ester, Rutin, and a New Steroid Sapogenin

By Ibrahim El-Sayed El-Kholy and Mohammed Abdel Monem Shaban

Two waxes and a ψ-taraxasteryl ester have been isolated from the light petroleum extract of the leaves of *Ficus carica*, L. Rutin, and a new trihydroxy-steroid sapogenin, ficusogenin, have been obtained from the ethanolic extract.

FROM the light petroleum extract, besides the furocoumarins and sterols previously isolated,[1] two waxes and a ψ-taraxasteryl ester were obtained. Wax A, $C_{60}H_{120}O_2$ or $C_{62}H_{124}O_2$, contains an ester carbonyl group as its infrared spectrum shows a carbonyl band at 1739 cm.$^{-1}$, while wax B is a hydrocarbon $C_{34}H_{70}$ or $C_{36}H_{74}$. Wax A resisted hydrolysis by either potassium hydroxide or sodium ethoxide.

After separation of the furocoumarins and waxes, the residue gave, on chromatography on alumina, besides β-sitosterol, a ψ-taraxasteryl ester. Its infrared spectrum is characterised by bands at: 2941s, 1730s, 1477m, 1374m, 1248s, 1027m, and 969w cm.$^{-1}$, of which the second is specific for an ester carbonyl.[2] On hydrolysis of this ester, ψ-taraxasterol, identified by direct comparison, and an acid, m. p. 64° (probably tiglic acid [3]), were obtained.

The concentrated ethanolic extract of the defatted leaves was separated into a water-insoluble fraction and a dark brown aqueous syrupy solution.

Rutin was obtained from the aqueous solution and its identity was established by direct comparison as well as by its infrared spectrum.[4] Moreover, on hydrolysis it gave quercetin, glucose, and rhamnose.

Quercetin was characterised by preparation of the 3,3′,4′,7-tetramethyl ether [5] by the action of diazomethane. However, the isomeric 3′,4′,5,7-tetramethylquercetin was obtained by methylation of the glycoside with diazomethane followed by hydrolysis.[6] Further methylation of either the 3,3′,4′,7-tetramethyl ether with dimethyl sulphate and alkali, or of the 3′,4′,5,7-tetramethyl ether with diazomethane gave the pentamethyl ether,[5] m. p. 153—154°.

From the water-insoluble fraction of the ethanol extract, a steroidal sapogenin, m. p. 296—298° (decomp.), was isolated, analysis of which indicated the formula $C_{27}H_{41}O_2(OH)_3$. It gave the general steroid colour reactions, but from differences in melting point it would appear not to be one of the known trihydroxysapogenins, digitogenin,[7] agavogenin,[8] nologenin,[8] tokorogenin,[9] agapanthagenin,[10] and cynarogenin,[11] and the

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name ficusogenin is provisionally suggested. The infrared spectrum of ficusogenin triacetate in carbon disulphide has bands at 2950s, 1748s, 1429s, 1361s, 1242s, 1168m, 1066s, 1042s, 985w, 965w, 954w, 925w, 909m, 843w, and 803w cm.$^{-1}$.

In the light of recorded physical properties,[12] particularly infrared absorption, ficusogenin appears to be a steroid isosapogenin having two of its hydroxyl groups in the 2- and 3-positions. The band at 1748 cm.$^{-1}$ is associated with the acetate carbonyl absorption, the 1361-cm.$^{-1}$ band is due to the methyl and methylene bending vibrations, and the bands at 1242, 1042, and 954 cm.$^{-1}$ are attributed to the presence of C-2 and C-3 acetyl groups. The simplicity of the band at 1242 cm.$^{-1}$, suggests a *trans*-fusion of rings A and B. On the other hand, the side-chain is associated with the bands at 985, 925, 909, and 843 cm.$^{-1}$ characteristic of the spiroketal structure. As the 909-cm.$^{-1}$ band is stronger than that at 925-cm.$^{-1}$, the *iso*-configuration of ring F is indicated.[13]

EXPERIMENTAL

The light petroleum used had b. p. 50—70°.

The Light Petroleum Extract.—Wax A. Wax A (11 g.) was obtained, after removal of the furocoumarins (12 g.) from the waxy deposit [1] (23 g.) on digestion with warm acetone. It was purified by repeated crystallisation from cyclohexane (charcoal) and then from benzene, as fine plates, m. p. 86—87° [Found: C, 82·2; H, 13·5%; *M* (Rast), 892. $C_{60}H_{120}O_2$ requires C, 82·5; H, 13·8%; *M*, 873·5. $C_{62}H_{124}O_2$ requires C, 82·6; H, 13·9%; *M*, 901·6]. This wax was recovered unchanged after being refluxed with ethanolic potassium hydroxide or sodium ethoxide. It does not decolourise bromine or potassium permanganate.

Wax B. The viscous yellowish-green oil (140 g.), left after separation of the furocoumarins and wax A, was diluted with acetone (200 ml.) and cooled to 0° when a greenish waxy substance (15 g.) separated. It was purified by repeated crystallisations from acetone then from ethyl acetate as glistening plates, m. p. 63—64° [Found: C, 85·3; H, 14·7%; *M* (Rast), 506. $C_{34}H_{70}$ requires C, 85·3; H, 14·7%; *M*, 478·9. $C_{36}H_{74}$ requires C, 85·3; H, 14·7%; *M*, 507]. It does not discharge the colour of bromine or potassium permanganate.

ψ-Taraxasteryl ester. The dark green acetone solution after isolation of wax B, was treated with charcoal and distilled. A solution of the resulting brown oily residue (10 g.) in light petroleum (50 ml.) was filtered through a column (14 × 3 cm.) of activated alumina (120 g.). Elution was with light petroleum (1 l.) then with benzene (750 ml.), 250-ml. fractions were collected. The yellowish oily residue (2·1 g.) recovered by evaporation of the light petroleum fractions, were dissolved in 25 ml. of light petroleum and rechromatographed on a column (11 × 2 cm.) of activated alumina (40 g.). Elution was with light petroleum (1 l.) which was collected in 4 equal fractions. A yellow oil (1·1 g.) was obtained from the first fraction and the crude ψ-taraxasteryl ester (0·8 g.), m. p. 190—210°, from the three other fractions. It was repeatedly crystallised from benzene–ethanol as plates, m. p. 237°, which depressed the m. p. of ψ-taraxasteryl acetate [1,10] [Found: C, 82·2; H, 11·1%; *M* (Rast), 460. $C_{35}H_{56}O_2$ requires C, 82·7; H, 11·0%; *M*, 512]. When this ester (0·1 g.) was heated under reflux in benzene (2 ml.) and 10% ethanolic potassium hydroxide (10 ml.) for 2 hr., a product, m. p. 186—190°, was obtained. ψ-Taraxasteryl acetate was obtained from this product by the action of acetic anhydride in presence of pyridine. After boiling with ethanol, the insoluble acetate was crystallised from benzene–ethanol as needles, m. p. 236—237°, not depressed by an authentic specimen [1,10] (Found: C, 81·8; H, 11·05. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%). Hydrolysis of this acetate with ethanolic potassium hydroxide in benzene gave ψ-taraxasterol, needles (from ethanol), m. p. and mixed m. p. 217° (Found: C, 84·6; H, 11·9. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%).

The alkaline solution left after separation of the product, m. p. 186—190°, from hydrolysis of the ψ-taraxasteryl ester was acidified with dilute sulphuric acid and repeatedly extracted with ether whereby an acid was obtained which crystallised from dilute methanol as plates, m. p. 64°.

β-*Sitosterol.* The first alumina column left after elution with benzene was divided from the top into two sections (10 and 4 cm.) which were separately extracted with boiling ethanol. The dark brown residue (1·3 g.) recovered from the second section yielded, on treatment with methanol, elongated plates, m. p. 130°. It crystallised from methanol as glistening plates,[1] m. p., and mixed m. p. 137°. It was further characterised by preparing its acetate, benzoate, and 3,5-dinitrobenzoate and comparing them with authentic specimens.[1]

The Ethanol Extract.—The defatted leaves (4 kg.) were extracted with boiling ethanol and the extract was then concentrated to a dark greenish-brown residue (430 g.). This residue was warmed with water (400 ml.) and the dark brown aqueous solution was separated by filtration. Finally, the water-insoluble part was extracted with hot ethanol which left on evaporation a dark greenish residue (70 g.).

Isolation of rutin. The dark brown aqueous syrupy solution (400 ml.) was freed from the greenish pigments by continuous ether extraction, and then kept at room temperature for 7—8 days when rutin separated as a brownish yellow precipitate (8 g.), m. p. 170—175°. It was purified by crystallisation from methanol as yellow needles, m. p. 180—182°. The homogenity of rutin was ascertained by giving only one spot when examined by paper chromatography on a Whatman No. 1 filter paper strip, using n-butanol–acetic acid–water (40 : 10 : 50) employing an ascending technique. Rutin was further crystallised from dilute methanol as canary yellow needles, m. p. 190—192°, not depressed on admixture with an authentic specimen (Found: C, 48·9; H, 5·65. Calc. for $C_{27}H_{30}O_{16},3H_2O$: C, 48·8; H, 5·5%), $[\alpha]_D^{23}$ −35·79° (*c* 1·062 in pyridine).

Hydrolysis of rutin. A solution of rutin (1 g.) in 3% methanolic sulphuric acid was heated on the water-bath for 1 hr. then concentrated and diluted. The aglycone, quercetin (0·5 g.), which separated as a canary-yellow precipitate, m. p. 300—310°, was filtered off, washed, and dried. It crystallised from dilute methanol as fine yellow needles, m. p. 314—315°, not depressed on admixture with an authentic specimen (Found: C, 59·2; H, 3·5. Calc. for $C_{15}H_{10}O_7$: C, 59·6; H, 3·3%).

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After fusion of quercetin with potassium hydroxide at 300°, phloroglucinol and protocatechuic acid were isolated and identified by m. p.s and mixed m. p.s Quercitin penta-acetate, pentabenzoate, 3,3′,4′,7-tetramethyl ether, 3,3′,4′,7-tetramethyl ether 5-acetate, 3′,4′,5,7-tetramethyl ether, and pentamethyl ether were also prepared. They all had m. p.s in agreement with those recorded and gave good analyses.

Identification of the sugar residue. The hydrolysate after separation of quercetin was neutralised with barium carbonate and demineralised by percolation through ion-exchange resins. The solution after evaporation in vacuum left a colourless sticky gum (0·4 g.). The occurrence of glucose and rhamnose in this sugar moiety was established by paper chromatography on Whatman No. 1 filter paper alongside glucose and rhamnose as controls. The solvent system used was n-butanol–ethanol–water (40 : 10 : 50) employing a descending technique and spraying with 10% aqueous ammonium molybdate.

Isolation of Ficusogenin.—The dark green water-insoluble residue (70 g.) of the ethanol extract was washed with light petroleum and with ether, and finally digested with boiling methanol. The sticky greenish-white residue (2 g.) left was purified by repeated crystallisations from benzene–ethanol (charcoal), when it was obtained as whitish micro-crystals, m. p. 280° (decomp.).

Purification was also effected by percolating a solution of the crude sapogenin (1 g.) in benzene–ethanol (1 : 1; 200 ml.) through a column (20 × 2 cm.) of activated alumina. Elution was with chloroform (500 ml.) which gave on evaporation traces of a waxy substance. Extraction of the alumina with boiling benzene–ethanol yielded, on concentration, the steroid sapogenin as clusters, m. p. 283—288° (decomp.).

Ficusogenin acetate was obtained when the sapogenol (1·5 g.) was heated with acetic anhydride (15 ml.) in presence of pyridine (15 ml.) for 3 hr. The crude acetate (1·7 g.) crystallised from benzene–ethanol as elongated needles, m. p. 171—172° [Found: C, 69·2; H, 9·1; Ac, 20·1%; *M* (Rast), 561. $C_{33}H_{50}O_8$ requires C, 69·0; H, 8·8; 3 Ac, 22·5%; *M*, 574]. The pure *ficusogenin* was prepared by hydrolysis of this acetate with ethanolic potassium hydroxide in presence of benzene. It crystallised from benzene–ethanol as plates, m. p. 296—298° (decomp.) (Found: C, 72·7; H, 10·2. $C_{27}H_{44}O_5$ requires C, 72·3; H, 9·9%).

Ficusogenin benzoate was prepared when a solution of the sapogenin (0·5 g.) in pyridine (10 ml.) was heated with benzoyl chloride (1·5 ml.) for 4 hr. It was recovered as usual, heated under reflux with methanol, and the insoluble portion crystallised from benzene–ethanol as needles, m. p. 199—200° (Found: C, 76·0; H, 7·7. $C_{48}H_{56}O_8$ requires C, 75·8; H, 7·4%). Hydrolysis of this ester yielded the original sapogenin, m. p. 296—298° (decomp.).

Ficusogenin p-*nitrobenzoate* was obtained when a solution of ficusogenin (0·5 g.) in pyridine (10 ml.) was heated with *p*-nitobenzoyl chloride (1·5 g.) for 4 hr. on a water-bath. The mixture was then poured into water, the solid separated, washed with sodium hydrogen carbonate solution and with water, boiled with methanol, and the ester filtered off hot. It crystallised from benzene–ethanol as pale yellow needles, m. p. 270—271° (Found: C, 64·85, 64·8; H, 6·1, 6·3; N, 4·8, 4·7. $C_{48}H_{53}N_3O_{14}$ requires C, 64·4; H, 6·0; N, 4·7%).

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Chemistry Department, Faculty of Science,
Alexandria University, Alexandria,
Egypt, U.A.R.

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