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CONSTITUENTS OF LOCAL PLANTS—IV.
FICUS CARICA L., *F. SYCOMORUS* L. AND *F. SALICIFOLIA* L. LEAVES

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Abstract—From the leaves of *Ficus carica* L. psoralene and psoralene and bergapten, in 0·37 and 0·59 per cent yield respectively, were isolated and in the unsaponifiable fraction of the fat β-sitosterol, β-amyrin and lupeol were identified. *Ficus sycomorus* L. leaves afforded only psoralene (0·9 per cent yield) and the unsaponifiable fraction of the fat was found to contain β-amyrin lupeol and β-sitosterol. The leaves of *Ficus salicifolia* L. contained psoralene and bergapten and in the unsaponifiable fraction of the fat lupeol and β-sitosterol.

THE world-wide interest[1] in the medical uses of xanthotoxin[2,3] and particularly psoralene[4,5] in the control of leucodermia (vitiligo) has led to a search for new natural sources, particularly of the latter compounds. It was early realized that the genus *Ficus* was promising as a likely source, and *Ficus carica* L. (common fig plant), *F. sycomorus* L. and *F. salicifolia* L. were therefore examined.

The ethanolic extract of the leaves was treated with cold alkali, thus converting the coumarins into sodium salts, and lipids removed by extraction with ether. The coumarins were freed by acid treatment and separated by chromatography on a silica gel column, the fractions obtained being examined by thin-layer chromatography on silica gel.[6] In using this technique the leaves of *F. carica* L., collected during October, gave both psoralene and bergapten in 0·37 and 0·59 per cent yield respectively on a dry weight basis.*

It is not unlikely that both the total coumarin content and the ratio of components might be different during other seasons. Rodighiero[8] has shown this occurs with Italian material. Psoralene has previously been identified in the same plant; but of Japanese origin by Okahara[9] and Fukushi.[10]

The ether extract was treated to give an unsaponifiable fraction which was resolved by chromatography on alumina. Two crystalline products were isolated and identified as β-sitosterol and β-amyrin. A third compound was identified as lupeol by thin-layer chromatography on silica gel.

* Soliman[7] has also isolated both coumarins in *F. carica* L. grown locally.

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[2] I. R. FAHMY and H. ABU-SHADY, *Quart. J. Pharm.* **20**, 281 (1947); **21**, 499 (1948); I. R. FAHMY, H. ABU-SHADY, A. SCHONBERG and A. SINA, *Nature* **160**, 468 (1947).
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[5] L. MUSAJO and G. RODIGHIERO, *Experientia* **18**, 153 (1962) and literature cited therein.
[6] For details, cf. F. M. ABDEL-HAY, E. A. ABU-MUSTAFA, B. A. H. EL-TAWIL and M. B. E. FAYEZ, *J. Chromatog.* In press (1964).
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[8] G. RODIGHIERO and C. ANTONELLO, *Farmaco* (*Parvia*), *Ed. Sci.* **14**, 679 (1959).
[9] K. OKAHARA, *Bull. Chem. Soc. Japan* **11**, 389 (1936); **13**, 635 (1938).
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Ficus sycomorus L. (syn. *Sycomorus antiquorum* Gasp.) is another large tree which produces an edible fig-like fruit. Surprisingly, nothing appears to be known about its chemical constitution. It is known that the milky sap is frequently used by Egyptian peasants as a local treatment for vitiliginous patches. When extracts of the leaves of this plant, collected during October, were treated in the same way as *F. carica* L. only one coumarin was isolated in a crystalline state and was identified as psoralene (0·19 per cent yield on dry weight). Examination of the unsaponifiable fraction of the fat removed from the leaves of *F. sycomorus* L. by chromatography on alumina led to the isolation of α-amyrin, lupeol and β-sitosterol.

From the leaves of *Ficus salicifolia* L., treated in the same way, psoralene and bergapten were obtained and from the unsaponifiable fraction lupeol and β-sitosterol were identified.

Throughout these studies considerable use was made of the technique of thin-layer chromatography on silica gel with benzene:acetone (9:1) or benzene:ethyl acetate (9:1) for coumarins,[6] and benzene:chloroform (9:1) for neutral triterpenoids and free sterols.

EXPERIMENTAL

Constituents of Ficus carica *L. Leaves*

The dried powdered leaves (680 g) were exhaustively extracted with alcohol and the concentrated extract (1 l.) was treated at room temperature with an equal volume of 10% alcoholic potassium hydroxide with occasional stirring. After dilution with water, the alkaline solution was repeatedly extracted with ether and the ether extract was washed with water till neutral, dried over anhydrous sodium sulphate, then evaporated to dryness to give 15 g of the fat-containing fraction *A* as a dark green mass. The alkaline layer was acidified with dilute hydrochloric acid then extracted with ether. The ethereal extract was successively washed with water, dilute sodium bicarbonate solution and then with water till neutral. After drying over anhydrous sodium sulphate the ether extract was evaporated to dryness to give 11 g of the free coumarin fraction *B*.

Fraction *A* (15 g) was saponified with 5% ethanolic potassium hydroxide (250 ml) under reflux for 2 hr. The unsaponifiable fraction was isolated in the usual manner as light greenish oil (4·5 g) and a portion (2 g) was chromatographed on alumina (200 g). Elution with petroleum ether removed 0·3 g of colourless wax and final elution with 3% methanol in benzene removed various fractions which were examined by thin-layer chromatography on silica gel G using benzene:chloroform (9:1) as solvent system and antimony trichloride as spray reagent. In this manner, a fraction (total weight 220 mg, R_f 0·27) was isolated which afforded β-amyrin as needles from methanol, m.p. and mixed m.p. 198–199°, $[\alpha]_D$ +87°. Acetylation (pyridine–acetic anhydride) afforded β-amyrin acetate, m.p. and mixed m.p. 238–240°. The i.r. absorption spectra (CS_2) of the two substances were identical in every detail. Another fraction (1·06 g) was removed which evidently contained two products and was thus resolved by fractional crystallization from methanol. This treatment gave β-sitosterol, R_f 0·13, m.p. and mixed m.p. 136–137°, $[\alpha]_D$ −36°; in the mother liquor, lupeol, R_f 0·26, was detected by comparison with authentic material.

One gramme of fraction *B* was chromatographed on a column of silica gel (120 g) and the process followed by thin-layer chromatography on silica gel G using benzene:acetone (9:1) as developing system and iodine solution as spray reagent. In this way it was possible to separate a fraction (260 mg, R_f 0·75) identified as psoralene, m.p. and mixed m.p. 168–171°. Another fraction (400 mg, R_f 0·77) was removed and identified as bergapten, m.p. and mixed m.p.

189–191°. The identities of both psoralene and bergapten were further confirmed by inspection of their i.r. spectra.

Constituents of F. sycomorus *L. Leaves*

One-half kilogramme of the dried powdered leaves was processed as described under *F. carica* L. to give 5·5 g of fraction *A* and 3·8 g of fraction *B*. The first fraction *A* was saponified with 5% alcoholic potassium hydroxide and worked up as usual to give 2·5 g of unsaponifiable matter. Treatment with acetic anhydride (8 ml) in pyridine (10 ml) followed by working up gave 2·7 g of acetylated material which was introduced on a 2 × 25 cm alumina column. Initial elution with petroleum ether removed 0·25 g of colourless waxy material. Elution with the same solvent removed a fraction which afforded, after crystallization from chloroform–methanol, 20 mg of α-amyrin acetate, m.p. and mixed m.p. 224–226°, $[\alpha]_D$ +85°. A fraction (0·85 g) was then removed and, found too complex to resolve by crystallization, was saponified with alcoholic potassium hydroxide and the hydrolysate (0·7 g) was chromatographed on 50 g alumina. After the removal of some waxy material, prolonged elution with benzene removed α-amyrin, m.p. and mixed m.p. 188–190°, $[\alpha]_D$ +81°, followed by 130 mg of lupeol, m.p. and mixed m.p. 210–213°. Benzoylation of the latter product gave lupeol benzoate, m.p. and mixed m.p. 265–268°. Final stripping of the second column with 1% methanol in benzene removed a small amount of β-sitosterol, m.p. and mixed m.p. 139–141°; $[\alpha]_D$ −35·5°. A crop of β-sitosteryl acetate was also removed from the first column by elution with benzene, and after crystallization from methanol it afforded 23 mg of pure material, m.p. and mixed m.p. 125–127°, $[\alpha]_D$ −38°. The identities of all these products were confirmed by examination of their i.r. absorption spectra.

One gramme of the coumarin fraction *B* was chromatographed on a 100 g silica gel column and benzene was used as eluent. Examination of the chromatogram on silica gel G thin layers, using benzene:acetone mixture (9:1) as developer, indicated that only one coumarin, R_f 0·75, was present. This was isolated (0·39 g) by crystallization from methanol and identified as psoralene, m.p. and mixed m.p. 168–171°. The i.r. absorption spectrum was identical with that of authentic psoralene.

Constituents of F. salicifolia *L. Leaves*

The dried powdered leaves (345 g) were extracted with alcohol and worked up as described under *F. carica* L. to give 7·5 g of fraction *A* and 2 g of fraction *B*.

Fraction *A* was saponified in the same manner as previously described to give 2·5 g of unsaponifiable matter which was acetylated (pyridine and acetic anhydride) and then chromatographed on 125 g alumina. Elution with petroleum ether first removed 429 mg of colourless waxy material and with 30% benzene in petroleum ether removed 340 mg of semi-solid substance which on crystallization from methanol gave lupeol acetate, m.p. and mixed m.p. 214–216°. A second fraction (128 mg), removed with the same eluent, was shown to be β-sitosteryl acetate, m.p. and mixed m.p. 125–128°. The course of chromatography was followed on thin layers of silica gel G using petroleum ether alone as developer.

The coumarin fraction *B* was chromatographed on 100 g silica gel. After preliminary washing with petroleum ether and with benzene, elution with 1% methanol in benzene gave a small amount of psoralene which was purified by sublimation under vacuum, m.p. and mixed m.p. 165–169°. The second product, removed with the same eluent, was crystallized from methanol and shown to be bergapten, m.p. and mixed m.p. 194–195°. The i.r. absorption spectra of both products were identical with those determined for authentic substances.