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The constituents of the seeds of *Asphodelus microcarpus* Viviani and *A. fistulosus* L.

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Evidence is presented that β-sitosterol and β-amyrin can be separated from the fixed oil of seeds of *A. microcarpus* Viviani and *A. fistulosus* L. by thin-layer, column and gas-liquid chromatography, together with other minor sterols (campesterol and stigmasterol). Myristic, palmitic, stearic, oleic and linoleic acids were identified in the oil of both species by thin-layer and gas-liquid chromatography. Sucrose, raffinose and stachyose were indicated in seeds of both species, also melibiose in seeds of *A. microcarpus*. Colchicine was not found in either species.

THE Mediterranean genus *Asphodelus* (family Liliaceae) is represented in Egypt by several species, two of the commonest being *A. microcarpus* Viviani and *A. fistulosus* L.

Limited investigations of the constituents of species of this genus have been made; Klein & Pollauf (1929) recorded evidence for the presence of colchicine in *A. albus* Willd., but this was refuted by Santavy (1956). van Rheede van Oudtshoorn (1964) detected anthraquinones (aloe-emodin, nataloe-emodin and chrysophanol) in several genera of the Liliaceae, including *A. albus* which contained chrysophanol and aloe-emodin as free anthraquinones; Tappi (1951) isolated flavoxanthin from this species. Khan, Qureshi & others (1961) isolated myristic, palmitic, stearic, oleic and linoleic acids from the seeds of *A. fistulosus* and showed that the unsaponifiable matter (1·8%) contained fucosterol and an unidentified yellow substance. Colin & Neyro (1931) found laevulosan, sucrose, a reducing sugar and an amorphous glucoside in tubers of *A. microcarpus* at the close of the vegetative period.

MATERIALS

The materials consisted of the dried ripe seeds of *A. microcarpus* Viviani, collected from the Western Mediterranean region at Burg Al Arab, 45 km west of Alexandria in June, 1965, and of *A. fistulosus* L., collected from the New Valley (oasis) in Upper Egypt in June, 1966. They were authenticated by Dr. K. H. Batanoumy, Botany Department, Faculty of Science, Cairo University.

Experimental and results

Phytochemical screening showed that seeds of both species contained fixed oil, carbohydrates (free sugars and mucilage), phenolic substances and alkaloids (Wagner reagent on acidified, solvent-free alcoholic extract) (Lewcowitch, 1921); flavonoids were absent (Bryant, 1950).

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SACCHARIDES

Carbohydrates were extracted from the defatted powdered (No. 40) seeds of each species with pyridine (Malpress & Morrison, 1949). The separation of the sugars was by ascending paper (Whatman No. 1) chromatography using n-butanol–acetic acid–water (40 : 10 : 50) for 16 hr; after air-drying, redevelopment was effected in the same solvent system for a further 16 hr. Spots were visualized with *p*-anisidine–phosphoric acid (Hough, Jones & Wadman, 1950). This procedure indicated the presence of stachyose, raffinose, melibiose and sucrose in the seeds of *A. microcarpus* and of stachyose, raffinose and sucrose in those of *A. fistulosus*.

FIXED OIL FRACTION

Seeds of *A. microcarpus* yielded 14·8% of oil; those of *A. fistulosus* 6·2% (mean of three determinations) after extraction with 2 litres of light petroleum (b.p. 40–60°) for 10 hr in a Soxhlet.

Preparation and fractionation of unsaponifiable matter. 100 g of each of the oils were separately saponified by refluxing (6 hr) with 0·5N ethanolic potassium hydroxide (1 litre). After concentration and dilution with water, the mixture was shaken with ether to complete extraction; ethereal extracts were washed until free from alkalinity and the ether distilled off. The yield of unsaponifiable, solvent-free residue was 1·03 and 0·98% from seeds of *A. microcarpus* and *A. fistulosus* respectively.

Approximately 1 g of unsaponifiable matter was dissolved in light petroleum (b.p. 40–60°) and eluted from a column packed with alumina in light petroleum with fraction (1) light petroleum (b.p. 40–60°) 500 ml; fraction (2) light petroleum–benzene (70 : 30) 750 ml; fraction (3) light petroleum–benzene (50 : 50) 1250 ml; fraction (4) benzene 100 ml; fraction (5) benzene–methanol (99 : 1) 100 ml; fraction (6) benzene–methanol (97 : 3) 500 ml.

Fractions (2), (3) and (6) gave positive tests for sterols which gave colour reactions with Lieberman-Burchard and Hagar Salkowski reagents. The results suggested the presence of β-sitosterol and β-amyrin in the seeds of each species; β-amyrin gave a pink-coloured ring with Lieberman-Burchard reagent, indicating a triterpenoid structure (Lewkowitch, 1921). Separation of β-sitosterol and β-amyrin from the combined fractions (3) and (6) was attempted by fractional crystallization from methanol; this gave a substance analogous with β-sitosterol contaminated with traces of β-amyrin (indicated by thin-layer chromatography on silica gel (Kieselgel G) first, whilst the mother liquor, after separation of "β-sitosterol", gave crystals shown to be β-amyrin with traces of "β-sitosterol". To ensure complete separation, alumina columns were used; elution with light petroleum (b.p. 40–60°) removed waxes and hydrocarbons, light petroleum–benzene (40 : 60) yielded needle-shaped crystals, shown to be pure β-amyrin after recrystallization from methanol, finally, light petroleum–benzene (50 : 50) yielded "β-sitosterol".

"β-Sitosterol", $C_{29}H_{50}O$, after several recrystallizations from methanol, melted at 137–138° (Heilbron & Bunbury, 1953a, reported 136–137°); the acetate, recrystallized from methanol, melted at 127–128° (reported 128°). Both melting points were undepressed when either the sterol or its acetate were mixed with authentic samples. The Rf of the sterol corresponded to authentic β-sitosterol in two different solvent systems. Found: C, 84·0, H, 12·0; calculated: C, 84·05, H, 12·1.

β-Amyrin, $C_{30}H_{50}O$, after recrystallization from methanol, melted at 198–199° (Heilbron & Bunbury, 1953b, reported 197–197·5°). Acetate m.p. 237°. Melting points of mixtures with authentic samples were undepressed. Found: C, 84·45, H, 11·7; calculated: C, 84·5, H, 11·7.

Although the values, determined experimentally, of various physicochemical constants (m.p., Rf and element analysis) of most plant sterols agree closely with published values, recent published data (Kallianos, Shelburne & others, 1963; Knights, 1965), has not infrequently shown them to consist of steroid mixtures. The β-sitosterol, after further purification by precipitation as steroid digitonide (Boughton & Wheatley, 1959), was therefore subjected to gas-liquid chromatography* and was shown to be a mixture of β-sitosterol, campesterol and stigmasterol.

FATTY ACIDS

Fatty acids of the two species were examined by thin-layer and gas-liquid chroatography, after conversion to their methyl esters (Burchfield & Storrs, 1962). The purified methyl esters were fractionated by converting the unsaturated esters into mercuric addition compounds (Jantzen & Andreas, 1959, 1961). The saturated esters were removed by thin-layer chromatography using silica gel and light petroleum (b.p. 60–80°)–ether (80:20); the derivatives of the unsaturated fatty esters were resolved using n-propanol–glacial acetic acid (100:1).

Thin-layer chromatography of the adducts showed only the presence of two zones corresponding to monoenes and dienes.

Saturated esters were eluted with a mixture of light petroleum (b.p. 60–80°)–ether (50:50). The saturated acid components were separated on silica gel G layers impregnated with undecane (Kaufmann & Makus,1960), developed with acetic acid–acetonitrile (1:1) and visualized with phosphomolybdic acid. The procedure indicated that the saturated acids in the oils of both species were myristic, palmitic and stearic.

* *Conditions for chromatography:* Apparatus: Pye Chromatograph "104". Column dimensions: glass column, 90 cm length and 0·25 cm diameter, U tube. Solid support: Celite gas chromosorb P 80/100 mesh. Stationary phase: Methyl substituted gum rubber (General Electric Co.) SE-30 (2·7%). Detector: Flame ionization. Carrier gas: Nitrogen, flow rate of 60 ml/min. Temperature: 260°; injection preheater, 280°. Recorder: Honeywell with chart speed of 1 inch/3 min. Sensitivity: 50×10^3. Sample size: 2–5 μl (containing 20–50 μg) of 1% solution of the sample in spectroscopic chloroform. n-Octacosane was added to all the samples, before injection, as internal standard.

Gas-liquid chromatography* of the methyl esters of the total fatty acids were effected and showed the presence of myristic, palmitic, stearic, oleic and linoleic acids.

ALKALOIDS

Initial screening revealed the presence of alkaloids in both species, but colchicine, colchamine and colcemid were shown to be absent by paper chromatography using Dragendorff reagent and by the failure of alkaloidal extracts to give any colour specific for colchicine (Smolenski, Crane & Voigt, 1958; Smith, Bullivant & Cox, 1963).

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* *Conditions for chromatography:* Apparatus: Pye Panchromatograph. Column dimensions: glass column 150 cm length and 0·25 cm diameter, U tube. Solid support: Celite gas chromosorb W/60/80 mesh (acid washed). Stationary phase: Polyethylene glycol adipate 10%. Detector: Argon detector at 550 V. Carrier gas: Argon at 60 ml/min. Temperature: 190°. Recorder: Honeywell with chart speed of 1 inch/3 min. Sensitivity: 10^{-8}. Sample size: 1 μl (50 μg) of 5% solution of the methyl esters in spectroscopic chloroform.