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STEROIDS AND TRITERPENOIDS OF GRAPEFRUIT (*Citrus paradisi*, Macfadyen). PART I

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Received March 9, 1955

The isolation of a steroidal and of a "sapogenic" constituent from the non-saponifiable fraction of the oil obtained from a Duncan variety grapefruit peel[1] was reported by Markley, Nelson, and Sherman (1). They assigned formula $C_{28}H_{48}O$ to the sterol and characterized the other product as a ketone of formula $C_{31}H_{52}O$ and m.p. 253–254°. However, the specific rotation of the sterol and the melting points of its derivatives did not correspond to any known substance. It was suggested, therefore, that the isolated compound was either a previously unidentified sterol or a mixture of plant steroids.

The procedure adopted by us for the isolation of these substances was similar to that of Markley, Nelson, and Sherman (1). The oil obtained by pressing of the peel was distilled under reduced pressure at 70–80° in order to separate the limonene, and then steam-distilled, whereby the volatile constituents were removed (2). The light petroleum extract of the residue was saponified, and the unsaponifiable part was dissolved in methanol. On concentration of the filtered solution, a crystalline precipitate, showing a strong Liebermann-Burchard reaction, was obtained. Purification by recrystallization and chromatography on alumina yielded a sterol with a formula $C_{29}H_{50}O$ and m.p. 139–140°. We have characterized this substance unequivocally as *22-dihydrostigmasterol* (*β-sitosterol*), by the preparation of its following derivatives: acetate, benzoate, dinitrobenzoate, and the hydrogenated acetate (see Table I). The identity of each of these derivatives was checked by a mixture m.p. determination with an authentic sample.

It is interesting to note that Swift (6) has isolated *β-sitosteryl glucoside* from the juice of *Citrus Aurantium sinensis*. The "sitosterol" of m.p. 139–140°, isolated by Matlack (7, 8) from the rind and the pulp of the same fruit, is probably also *β-sitosterol*.

A second portion of grapefruit peel-oil was examined for other possible components. In this case, the total unsaponifiable fraction obtained as before was caused to crystallize partially by treatment with a little methanol, and the oily crystalline precipitate was chromatographed on alumina. The first fractions, collected by elution with a pentane-benzene mixture, consisted of crystals melting

[1] The botanical name of the Duncan variety of grapefruit given by these authors is *Citrus grandis*, Osbeck, which is synonymous with *Citrus decumana*. The latter name was used by Zoller (2) and Wehmer (3) to describe the common grapefruit which belongs to the Duncan variety. According to the classification of Swingle (4) and Guentner (5) the correct name of the common grapefruit (Duncan variety) is *Citrus paradisi*, Macfadyen. The names of *Citrus grandis* or *Citrus decumana* are used for the pummelo fruit (4).

TABLE I

COMPARISON OF STEROLS AND STEROL DERIVATIVES

Compound	22-Dihydrostigmasterol[a]		*C. Paradisi* Sterol	
	M.P., °C.	$[\alpha]_D^\circ$	M.P., °C.	$[\alpha]_D^\circ$
Sterol	140	−37	139–140	−35
Steryl acetate	127–128	−42	129–130	−43
Steryl benzoate	146–147	−14	146–147	−12
Steryl *m*-dinitrobenzoate	202–203	−10	200–201	−10
Stanol acetate	137–138	+14	136–137	+14

[a] Data from Elsevier's Encyclopedia of Organic Chemistry, Elsevier Publishing Co., Inc., New York, Vol. 14 (1940).

TABLE II

COMPARISON OF TRITERPENOID KETONES AND THEIR DERIVATIVES

Compound	Friedelin		*C. Paradisi* Ketone		Sapogenic Ketone[e]	
	M.P., °C.	$[\alpha]_D^\circ$	M.P., °C.	$[\alpha]_D^\circ$	M.P. °C.	$[\alpha]_D^\circ$
Ketone	255–261[a] 248–250[c] 264–265[c, f] 262–263[d, f]	−29[a] −28[c] −21[d]	256–257 267–268[f]	−19	253–254	−20
Enol benzoate	255–262[a] 246–249[c] 265–266[c, f]	+66[a] +64[c]	255–256 266–267[f]	+59		
Oxime	290–294[b]		280–282 (dec) 287–289[f] (dec)	+56	281–282	

[a, b, c, d, e] See references (9), (10), (11), (12), and (1) respectively. [f] These melting points were determined in evacuated tubes.

at 60° which were not further examined, and are probably paraffins identical with those isolated from grapefruit peel oil by former investigators (1). Further elution with the same solvent mixture gave fine needles which, after purification by repeated recrystallization melted at 256–257°. This substance was identified as the saturated triterpenoid ketone *friedelin* (Table II), a compound previously isolated from cork (9–11) and, lately, from lichens (12). A comparison of the infrared spectra of the isolated ketone with an authentic sample[2] showed complete identity.[3]

[2] We are indebted to Dr. O. Jeger of the Federal Institute of Technology, Zurich, Switzerland, for a sample of this compound.

[3] We are grateful to Prof. D. Ginsburg of the Haifa Institute of Technology, Israel, for the infrared spectra (Perkin-Elmer Model 12 C spectrometer with sodium chloride prism).

EXPERIMENTAL[4]

Isolation of the sterol. Oil from the peel of grapefruit[5] (5 kg.) was distilled under reduced pressure (25–28 mm.) at 75–76°. The distillate (4.5 kg.) consisted of crude limonene. The residue then was steam-distilled and the remaining nonvolatile oil was separated from the water by ether extraction. It then was heated under reflux for one hour with light petroleum (1.5 l.), and the solution was filtered and evaporated to dryness. The residue (196 g.) was heated on a steam-bath with 2 l. of ethanolic potassium hydroxide (4%) and 100 cc. of benzene for two hours. The solution then was concentrated *in vacuo* to a 1-l. volume. Then 5 l. of water was added and the unsaponifiable part (109 g.), isolated by means of ether, was dissolved in 2 l. of hot methanol. Upon concentration of this solution to 1.1 l., 15.7 g. of oil precipitated. This was separated, and the solution was further concentrated to 300 cc. and left overnight at 0°. The crystals produced (17 g.), showing a strong Liebermann-Burchard reaction, were recrystallized twice from ether-methanol to yield 9 g. of plates, m.p. 131–132°. These crystals were dissolved in 50 cc. of a pentane-benzene (4:1) mixture and were chromatographed on a column (3.2 cm. x 7.5 cm.) of 220 g. of ethyl acetate-washed alumina. From this, 3.2 l. of benzene-ether (9:1) eluted 4.0 g. of crystals, m.p. 136–138°, $[\alpha]_D$ −30°, which, after five recrystallizations from ether-methanol melted at 139–140°, $[\alpha]_D$ −35°. No depression of the melting point was observed on mixing with an authentic sample of *22-dihydrostigmasterol.*

The *steryl acetate* was prepared by treating 1 g. of the *sterol* with 10 cc. of acetic anhydride and 10 cc. of pyridine and keeping the mixture overnight at room temperature. Addition of water, isolation by means of ether, and five recrystallizations from ether-methanol yielded needles of m.p. 129–130°, $[\alpha]_D$ −44°.

Anal. Calc'd for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48.
Found: C, 81.48; H, 11.43.

The m.p. was not depressed on admixture with *22-dihydrostigmasteryl acetate.*

The *steryl benzoate.* The *sterol* (200 mg.) was heated with 0.5 cc. of pyridine and 2 cc. of benzoyl chloride for 30 minutes on a steam-bath. Water was added, and the product was filtered off and washed with hot water.

Three recrystallizations from methylene chloride-methanol furnished plates, m.p. 146–147°, $[\alpha]_D$ −12°.

Anal. Calc'd for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49.
Found: C, 83.10; H, 10.44.

No depression of m.p. was observed on mixing with *22-dihydrostigmasteryl benzoate.*

The *steryl m-dinitrobenzoate.* The *sterol* (250 mg.) was heated with 5 cc. of pyridine and 1 g. of dinitrobenzoyl chloride for two hours on a steam-bath. Water was added, and the product was isolated by means of ether and recrystallized thrice from methylene chloride-ethyl acetate to give yellow plates, m.p. 200–201°, $[\alpha]_D$ −11°.

Anal. Calc'd for $C_{36}H_{52}N_2O_6$: C, 71.02; H, 8.61.
Found: C, 70.95; H, 8.53.

The m.p. was not depressed on admixture with *22-dihydrostigmasteryl m-dinitrobenzoate.*

The *stanol acetate.* The *steryl acetate* (100 mg.) in 30 cc. of acetic acid was hydrogenated with 50 mg. of platinum oxide. One mole of hydrogen was absorbed. The catalyst was filtered off and the solvent was evaporated under reduced pressure. The product was dissolved in 15 cc. of carbon tetrachloride and treated with 5 cc. of acetic anhydride and 5 cc. of concentrated sulfuric acid. After 1 cc. of water had been added the colored acidic layer

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[4] Melting points are corrected. Rotations were determined at 18° in chloroform solutions. We are indebted to Mr. Avraham Aroio for his technical assistance. The microanalyses were carried out by Mr. W. Manser at the Federal Institute of Technology, Zurich, Switzerland.

[5] Purchased from "Mizaron Ltd." Kfar-Saba, Israel.

was separated and the carbon tetrachloride solution was washed with water. The product was recrystallized from ether-methanol to give needles, m.p. 135–136°, $[\alpha]_D$ +14°.

Anal. Calc'd for $C_{31}H_{54}O_2$: C, 81.16; H, 11.87.
Found: C, 81.17; H, 11.80.

A mixture m.p. with *stigmastanol acetate* showed no depression.

Isolation of the ketone. Grapefruit peel oil (30 kg.) was distilled under reduced pressure, and the residue was steam-distilled and the non-volatile part (2240 g.) was extracted with light petroleum. The petroleum was distilled off, leaving a residue (1880 g.) which was heated under reflux with 10 l. of methanol to give a dark red solution (A) and a resinous residue (B). Then 300 g. of potassium hydroxide in 1 l. of methanol was added to the solution (A) and the whole was heated under reflux for 2 hours. The resinous residue (B) was hydrolyzed with a mixture of 5 l. of methanol, 1 l. of benzene, and 150 g. of potassium hydroxide. Both unsaponifiable parts, isolated with ether, were combined and heated on a steam-bath with 6 l. of methanol; the solution was filtered free from insoluble material and concentrated to a 3-l. volume. The precipitated, partly crystalline material (99 g.), was collected, dried, and suspended with 1 l. of pentane, and chromatographed on a column (5.5 cm. x 52.5 cm.) of 1 kg. of alumina. Successive elution with pentane-benzene mixture (9:1—5 l., 4:1—2.7 l., and 2:1—0.7 l.) furnished 6.1 g. of crystals, m.p. 58–60°. The substance was not effected by concentrated sulfuric acid at 100°, did not give a color reaction with tetra-nitromethane. and did not show any selective absorption in the ultraviolet. An analytical sample was obtained by two recrystallizations from ether-methanol (m.p. 60–62°).

Anal. Calc'd for $C_{29}H_{60}$: C, 85.20; H, 14.80.
Calc'd for $C_{31}H_{64}$: C, 85.23; H, 14.77 (1).
Found: C, 85.35; H, 14.80.

Further elution with pentane-benzene mixtures (1:1—2.35 l.) yielded 7.4 g. of long needles, which, after two recrystallizations from methylene chloride–ethyl acetate, gave 2.6 g. of friedelin, m.p. 256–257° (267–268° in *vacuo*), $[\alpha]_D$ +19, ν_{max}^{KBr} 1709 cm.$^{-1}$ (saturated ketone).

Anal. Calc'd for $C_{30}H_{50}O$: C, 84.44; H, 11.81.
Found: C, 84.37; H, 11.78.

No depression of m.p. was observed when mixed with an authentic specimen of *friedelin*.

The *enol benzoate.* The *ketone* (300 mg.) and 3 cc. of freshly distilled benzoyl chloride were heated for 2 hrs. at 170–195°, cooled to room temperature, and treated with 6 cc. of ethanol. The mixture was heated for 10 minutes on a steam-bath, and the crystals were collected, washed with small portions of hot ethanol, and recrystallized thrice from chloroform-ethyl acetate to yield needles of m.p. 255–256°, (266–267° in *vacuo*), $[\alpha]_D$ +59°.

Anal. Calc'd for $C_{37}H_{54}O_2$: C, 83.72; H, 10.25.
Found: C, 83.78; H, 10.24.

The mixture m.p. with *friedelin enol benzoate* showed no depression.

Oxime. The ketone (200 mg.), dissolved in a mixture of 100 cc. of ethanol and 100 cc. of benzene, was treated with 200 mg. of hydroxylamine hydrochloride and 5 cc. of pyridine. After heating under reflux for 2 hours, the substance was isolated by means of ether and was recrystallized from chloroform and ethanol to yield prisms of m.p. 280–282° (dec.), (287–289° dec., in *vacuo*), $[\alpha]_D$ +56°.

Anal. Calc'd for $C_{30}H_{51}NO$: C, 81.57; H, 11.64.
Found: C, 81.25; H, 11.45.

No depression of m.p. was observed when the substance was mixed with *friedelin oxime*.

SUMMARY

The peel oil of grapefruit, *Citrus paradisi*, Macfadyen has been found to contain *22-dihydrostigmasterol* (*β-sitosterol*) and the saturated pentacyclic triterpenoid ketone *friedelin*.

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