g., b.p. 96–120° (1.2–1.8 mm.), n_D^{20} 1.4402. The distillation was stopped at this point because of decomposition. The residue was tarry. Fractions (1), (2), and (3) were combined and redistilled through a 6-in. Vigreux column to give the following fractions: (*1*) 4.6 g., b.p. 57–75° (0.7 mm.), n_D^{20} 1.4250 (diethyl acetylphosphonate); (*2*) 0.1 g., b.p. 75–84° (0.7 mm.), n_D^{20} 1.4322; (*3*) 1.2 g., b.p. 86–106° (0.7 mm.), n_D^{20} 1.4420; (*4*) 2.2 g., b.p. 109–112° (0.7–0.8 mm.), n_D^{20} 1.4430.

Anal. Calcd. for $C_8H_{15}O_5P$: C, 43.24; H, 6.80; P, 13.94. Found on fraction (3): C, 43.78; H, 7.13; P, 14.33.

The infrared spectra of fractions (*3*) and (*4*) indicated the presence of both diethyl 1-acetoxyvinylphosphonate and the β-lactone of 3-diethylphosphono-3-hydroxybutyric acid in addition to traces of diethyl acetylphosphonate.

Diethyl 1-acetoxyethylphosphonate (VI). (*a*) *From diethyl 1-acetoxyvinylphosphonate.* Diethyl 1-acetoxyvinylphosphonate (11.1 g., 0.05 mole) dissolved in 50 ml. of absolute ethyl alcohol was placed in a pressure bottle, and 5 g. of a suspension of Raney nickel in ethyl alcohol was added. Hydrogen was added until the pressure reached 45 p.s.i., and the temperature was raised to 57°. The reaction was continued at this temperature until 0.05 mole of hydrogen had been absorbed (2.5 hr.). The Raney nickel was removed by filtration and 0.2 g. of anhydrous sodium carbonate was added to the filtrate. The solution was then distilled *in vacuo* through a 6-in. Vigreux column. After the forerun had been removed up to a head temperature of 101° (3.0 mm.), 4.0 g. (35.7%) of diethyl 1-acetoxyethylphosphonate was collected at 101–102° (3.0 mm.), n_D^{20} 1.4265.

Anal. Calcd. for $C_8H_{17}O_5P$: C, 42.85; H, 7.64. Found: C, 42.47; H, 7.90.

The infrared spectrum of this sample of diethyl 1-acetoxyethylphosphonate was identical with that of this ester prepared by other methods.[9]

(*b*) *From diethyl 1-hydroxyethylphosphonate.* Acetic anhydride (40.8 g., 0.4 mole) and diethyl 1-hydroxyethylphosphonate[9] (36.4 g., 0.2 mole) were dissolved in 100 ml. of pyridine, and the solution was stirred for 24 hr. The reaction mixture was distilled *in vacuo* through a 6-in. column packed with glass helices. After the pyridine and forerun up to a temperature of 94° (1.8 mm.) had been removed, 26 g. (58%) of diethyl 1-acetoxyethylphosphonate was collected at 94–96° (1.8 mm.), n_D^{20} 1.4265.

Anal. Calcd. for $C_8H_{17}O_5P$: C, 42.85; H, 7.64; P, 13.82. Found: C, 43.09; H, 7.68; P, 14.04.

The infrared spectrum of this product was identical with that of this ester prepared by other methods.[9]

KINGSPORT, TENN.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE]

Steroids and Triterpenoids of Citrus Fruit. II. Isolation of Citrostadienol[1]

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Grapefruit peel oil was found to contain citrostadienol, a new doubly unsaturated steroidal alcohol. The isolation of this compound as well as of β-sitosterol and friedelin from orange peel oil is also reported.

In the first paper of this series we described the isolation of β-sitosterol and friedelin from grapefruit peel oil. Further investigation of the content of the nonvolatile unsaponifiable fraction of this oil has now revealed the presence of an additional compound called by us citrostadienol, $C_{30}H_{50}O \pm CH_2$,[2] m.p. 162–164°, $[\alpha]_D$ +24°.

This compound was first found in the β-sitosterol mother liquors. Later, it could be isolated directly by careful chromatography on alumina of the total crystalline material obtained from the unsaponifiable fraction of the peel oil. The quantity of the isolated citrostadienol amounted to only *ca.* 0.01% of the total. In order to obtain more of this compound large quantities of grapefruit peel oil were needed. Due to the difficulty in obtaining such quantities of this peel oil the more readily accessible orange peel oil was examined for its content of citrostadienol.

The isolation of a steroid from sweet orange (*Citrus Aurentium sinesis*) peel oil was reported already in 1900. Stephan[3] obtained from the peel of Italian sweet oranges a compound with m.p. 138° giving a Liebermann-Burchardt color.[4] A phytosterol with similar constants was isolated by Naves from Guinea oranges.[5] Matlack in an extensive study of the constituents of California orange peel oil succeeded in isolating two phytosterols melting at 139° and 150°, respectively (the acetates melted at 128° and 113.5–114°, respectively), and a "phytosterylin".[6] The compound with m.p. 139° later referred to as "sitosterol" possesses the physical constants of β-sitosterol, as is the case with the compounds isolated by Naves[5] and by Stephan.[3] The "phytosterylin" is most probably β-sitosterol glycoside.[7] This glycoside was found recently to be a constituent of orange juice.[8]

(1) Presented in part at the 18th Meeting of the Chemical Society of Israel, 1955 (*cf. Bull. Res. Coun. Israel*, **5A**, 105 (1955). For Part I, see "Steroids and Triterpenoids of Grapefruit," Weizmann, Meisels, and Mazur, *J. Org. Chem.*, **20**, 1173 (1955).

(2) The distinction between the C_{29}, C_{30}, and C_{31} formulations cannot be made on the basis of the molecular weight determination by the *Rast* method or by C, H analyses.

(3) Stephan, *J. prakt. Chem.*, **62**, 523 (1900).

(4) The molecular formula given for this compound, $C_{28}H_{48}O_2$, undoubtedly included water of crystallization. It is known that the plant 3β-hydroxy steroids may contain water of crystallization, which is removed only with difficulty.

(5) Naves, *Parfums France*, **10**, 181 (1932).

(6) Matlack, *J. Am. Pharm. Assoc.*, **18**, 24 (1928).

(7) Matlack, *J. Org. Chem.*, **5**, 104 (1940).

(8) Swift, *J. Am. Chem. Soc.*, **74**, 1099 (1952).

For examination of the orange (*Citrus Aurentium sinensis*) peel oil we used the procedure adopted previously for the grapefruit peel oil.[1] The non-volatile part of the orange oil was saponified and the unsaponifiable part treated with methanol. Concentration of the methanolic solution gave a crystalline precipitate, which was chromatographed on alumina. From the eluted fractions we isolated successively the following compounds: paraffins, friedelin, cerylalcohol, citrostadienol and β-sitosterol. Paraffins and ceryl alcohol have already been isolated and identified by former investigators.[6] Friedelin and β-sitosterol were identified by us by comparison with authentic samples.[1] The fractions which were eluted between pure citrostadienol and β-sitosterol gave mixed crystals of these two compounds, m.p. 150°, $[\alpha]_D$ ±0°, which could not be separated by crystallization. Acetylation of this material gave crystals with the constant m.p. 115°, $[\alpha]_D$ 10°. The separation of this mixture could be effected only through rechromatography on alumina. It is probable that the phytosterol melting at 150°, isolated by Matlack,[6] consists of a mixture of β-sitosterol and citrostadienol.

Citrostadienol gives a precipitate with digitonin. It forms a monoacetate, m.p. 145–146°, $[\alpha]_D$ 39°, and on oxidation yields a ketone, m.p. 147–148°, $[\alpha]_D$ 15° $\nu_{max.}$ 1715 cm^{-1}, no absorption maxima in the ultraviolet above 218 mμ, from which the alcohol can be regenerated by reduction with lithium aluminum hydride. Citrostadienol gives a violet-blue-green Liebermann-Burchardt coloration and reacts positively in the Tortelli-Jaffe reaction[9] and Fieser's selenium dioxide test.[10] It absorbs one molar equivalent of hydrogen on hydrogenation over platinum in acetic acid. If the above hydrogenation is carried out in the presence of hydrochloric acid, the substance absorbs two molar equivalents of hydrogen giving a fully saturated product.

The above described observations suggest that citrostadienol is a doubly unsaturated 3β-hydroxy steroid, one of the double bonds being located in the $\Delta^{7,8}$ or $\Delta^{8,9}$-position. On the other hand, the molecular rotation differences between citrostadienol, its acetate, and the corresponding ketone, resemble those of the tetracyclic triterpenes[11] rather than the steroids. It is interesting to note that the "α-sitosterols," which accompany β-sitosterol in other plants, show a similarity to citrostadienol in this respect.[12] The elucidation of the structure of citrostadienol is now in progress.

(9) *Cf.* Fieser and Fieser, *Natural Product Related to Phenanthrene*, third ed., Reinhold Publishing Co., 1949, pp. 100–101.

(10) Fieser, *J. Am. Chem. Soc.*, **75**, 4395 (1953).

(11) Barton, *J. Chem. Soc.*, 813 (1945).

(12) *Elsevier's Encyclopedia of Organic Chemistry*, Vol. 14 Supplement, Triterpenes, 1952, pp. 1307–10.

EXPERIMENTAL[13]

Isolation of citrostadienol from grapefruit peel oil. A. The The oil from the peel of grapefruit was treated as described in part I[1] yielding 17 g. of unsaponifiable crystalline product. This was crystallized twice from ether-methanol, the crystals were collected, and the mother liquors (8 g.) were combined, concentrated, and left for a few days in the cold. The crystals thus produced had m.p. 153–155° and gave positive Tortelli-Jaffe and Fieser selenium dioxide tests. Five crystallizations from ether-methanol afforded pure citrostadienol, m. p. 162–164° (*in vacuo* 167–168°), $[\alpha]_D$ +24°.

Anal. Calcd. for $C_{29}H_{48}O$: mol. wt., 413; C, 84.40; H, 11.72; for $C_{30}H_{50}O$: mol. wt., 427; C, 84.44; H, 11.81; for $C_{31}H_{52}O$: mol. wt., 441; C, 84.48; H, 11.89. Found: mol. wt., 450; C, 84.64; H, 11.61.

B. The unsaponifiable crystalline material (19 g.) obtained from another quantity of peel oil (6 kg.) was dissolved in 150 cc. of pentane-benzene mixture (9:1) and chromatographed on a column of 600 g. of alumina. The first fractions eluted with 3 l. of a mixture of pentane:benzene (9:1) were discarded. The next fraction eluted with 600 cc. of a benzene-ether mixture (9:1) gave 610 mg. of crystals, m.p. 153–156°, which after additional 5 crystallizations from ether-methanol gave plates, m.p. 160–162°, undepressed on admixture with the citrostadienol mentioned above. The fraction eluted next gave 900 mg. of plates, m.p. 142–148°, which after three crystallizations showed m.p. 148–150°, $[\alpha]_D$ ±0°. The melting point of this material could not be increased by additional crystallizations. Acetylation of this material with pyridine and acetic anhydride gave crystals of the acetate, m.p. 115°, $[\alpha]_D$ +10°. On hydrolysis with methanolic potassium hydroxide the starting material with m.p. 148–150° was recovered.

Further elution with 4 l. of benzene-ether (9:1) gave crystals, m.p. 139°, identified as β-sitosterol (mixture melting point, infrared spectrum).

Five hundred milligrams of the material with m.p. 148–150° was rechromatographed on alumina (15 g.). The fraction eluted with benzene-ether (9:1) gave crystals (150 mg.), m.p. 154–156°. Crystallization from ether-methanol gave citrostadienol, m.p. and mixed m.p. 162–164°. The fraction eluted next with the same solvent mixtures gave 200 mg. of β-sitosterol m.p. and mixed m.p. 139–140°.

Isolation of citrostadienol from orange peel. One kilogram of concentrated orange oil was distilled under reduced pressure at 80–120° (12–15 mm.).[14] The distillate consisted of limonene (450 g.). The rest was further distilled in high vacuum at 90–120° (0.5 mm.). The brown residue (460 g.) was then saponified with 2 l. of methanolic potassium hydroxide (3%) on the steam bath. The solution was concentrated *in vacuo* to a third of its volume, 3 l. of water was added and the neutral part extracted with ether. The ethereal extract was washed several times with water, dried, and evaporated. The dark unsaponifiable part was triturated with 2 l. of methanol and left in cold overnight. The crystalline product obtained (18.3 g.) was collected, dissolved in pentane, and chromatographed on alumina (6.00 g.).

The first fraction, eluted with 1400 cc. of pentane-benzene (9:1) mixture, gave 1.4 g. of crystals, m.p. 60–65°. This

(13) Melting points are uncorrected. Rotations were measured at 20° in chloroform solution in a 1 dm. tube and a concentration of 10 ± 2 mg./cc. Infrared spectra (Baird double-beam) were determined in chloroform solution. The microanalyses were carried out in our microanalytical department under the direction of Mr. E. Meier. We are indebted to Miss Rivka Shapira for her helpful technical assistance.

(14) We are indebted to the Citrus Products Manufacturers' Association, Tel-Aviv, Israel, for supplying us with this oil. It was obtained by concentration of the orange peel oil *in vacuo* and subsequent treatment with 80% ethanol.

material did not give a coloration with tetranitromethane, did not possess absorption in the ultraviolet, resisted treatment with hot sulfuric acid, and consisted of paraffins.[6]

The next fractions, eluted with 400 cc. of pentane-benzene (1:1) gave 500 mg. of needles, m.p. 223–250°. Crystallization from ethyl acetate and sublimation in high vacuum gave friedelin, m.p. 256–257°, $[\alpha]_D$ +20°.

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.35; H, 11.70.

No depression of melting point was observed when mixed with an authentic specimen of friedelin. A comparison of the infrared spectra of this compound with an authentic sample showed complete indentity.

Further elution with 400 cc. of pentane-benzene (2:1) yielded a waxy material (550 mg.), m.p. 77–79°. This compound gave no coloration with tetranitromethane and did not show any appreciable absorption in the ultraviolet. The infrared spectrum possessed a hydroxyl band. It is probably identical with the ceryl alcohol previously isolated by Matlack from orange peel oil.[6]

Anal. Calcd. for $C_{26}H_{54}O$: C, 81.60; H, 14.20. Found: C, 81.85; H, 14.34.

The next fraction (1.4 g.), eluted with 500 cc. of benzene and 500 cc. of benzene-ether (9:1) gave crystals, m.p. 145–157°.

The last fraction of the chromatogram, eluted with 3 l. of benzene-ether (1:1) gave 5.5 g. of crystals, m.p. 132–138°. After crystallization from methanol the substance showed m.p. 139–140° and gave no depression on admixture with β-sitosterol. The infrared spectra of the two compounds showed complete identity.

The fractions with m.p. 145–157° were rechromatographed on 50 g. of alumina. Successive elution with 150 cc. of pentane-benzene (1:1) and 300 cc. of pentane-benzene (1:2) gave respectively 340 mg. of crystals with m.p. 157–161° and 930 mg. with m.p. 148–154°. The material with m.p. 157–161° was crystallized twice from ether-methanol to give citrostadienol, m.p. and mixed m.p. 162–164° $[\alpha]_D$ +24°. The material with m.p. 148–154° after another chromatography and crystallizations gave an additional 260 mg. of citrostadienol.

Citrostadienol acetate. Citrostadienol (200 mg.; m.p. 162–164°) was treated with 2 cc. of pyridine and 2 cc. of acetic anhydride and left overnight at room temperature. Isolation with ether and crystallization from absolute methanol gave citrostadienol acetate, m.p. 142–143° (145–146° *in vacuo*), $[\alpha]_D$ +45°.

Anal. Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08; $C_{32}H_{52}O_2$: C, 81.99; H, 11.18; $C_{33}H_{54}O_2$: C, 82.09; H, 11.27. Found: C, 81.73; H, 11.10.

Hydrolysis of the acetate. The acetate (100 mg.) was saponified with 100 cc. of methanolic potassium hydroxide (3%). Isolation from ether gave plates, m.p. 162–164°. No depression of melting point was observed when mixed with citrostadienol.

Oxidation of citrostadienol. A. Citrostadienol (150 mg.; m.p. 162–164°) was dissolved in 15 cc. of toluene, 5 cc. of cyclohexanone was added, and the mixture was distilled until 10 cc. of distillate had been collected in order to remove water. One gram of aluminum isopropoxide dissolved in 10 cc. of toluene was added dropwise to the boiling solution during 5 min., and the reaction mixture was refluxed for another 45 min. It was then cooled, water was added, and the solvents were removed by steam distillation. The resulting solid was collected by filtration and extracted with chloroform. Evaporation of the solvent gave a crystalline residue which was chromatographed on alumina (5 g.). Elution with pentane-benzene (9:1) gave 80 mg. of citrostadienone, which after crystallization from methanol showed m.p. 146–147°, $[\alpha]_D$ +15°.

Anal. Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29; $C_{30}H_{48}O$: C, 84.84; H, 11.39; $C_{31}H_{50}O$: C, 84.86; H, 11.49. Found: C, 84.61; H, 11.51.

B. A solution of 200 mg. of citrostadienol in 4 cc. of dry pyridine was added to a solution of chromium trioxide (200 mg.) in 4 cc. of pyridine. After being allowed to stand overnight at room temperature, water was added and the material was isolated with ethyl acetate. The residue (190 mg.) was chromatographed on 6 g. of alumina. The fraction eluted with pentane gave 85 mg. of crystals, m.p. 146–147°, $[\alpha]_D$ +15°, undepressed on admixture with the citrostadienone obtained above.

Citrostadienol from citrostadienone. A solution of 100 mg. of citrostadienone in 10 cc. of ether was added to 200 mg. of lithium aluminum hydride in 20 cc. of ether. After being refluxed for 1 hr., the mixture was decomposed with dilute sulfuric acid and ice. The material was isolated with ether and crystallized from ether-methanol to give 80 mg. of citrostadienol, m.p. and mixed m.p. 162–164°.

Hydrogenation of citrostadienol. A. Citrostadienol (30 mg.) in 10 cc. of acetic acid was hydrogenated in the presence of 10 mg. of platinum oxide. One molar equivalent of hydrogen was absorbed. The solid product showed coloration with tetranitromethane, gave positive Lieberman-Burchardt and Tortelli-Jaffe tests, and did not react in Fieser's selenium dioxide reaction.

B. Two drops of concentrated hydrochloric acid were added to a solution of 25 mg. of citrostadienol in 10 cc. of acetic acid and the solution was hydrogenated over 10 mg. of platinum oxide. Two molar equivalents of hydrogen were absorbed. The solid product showed no unsaturation in the tetranitromethane and Lieberman-Burchardt tests.

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