trace of glucose and a strong spot corresponding to D-fructose. When this sirup was dissolved in hot absolute ethanol and cooled, crystalline D-fructose separated, $[\alpha]^{25}D$ −87°, equilibrium value in water (*c* 3.5), m.p. 104–107°. No attempt was made at this time to obtain a high yield of crystalline D-fructose. The fact that D-fructose crystallized from the crude sirup can be taken as evidence that the yield could be greatly increased by further manipulation.

DIVISION OF AGRICULTURAL BIOCHEMISTRY
UNIVERSITY OF MINNESOTA
ST. PAUL, MINN.

Isolation of Campesterol and Δ^7-Stigmastenol from Rye Germ Oil[1]

BY H. A. SCHUETTE AND W. E. LINK

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Campesterol has been isolated previously from wheat germ oil, rape-seed oil and soy bean oil, and its constitution has been elucidated.[2,3] Δ^7-Stigmastenol has been shown to be a constituent of wheat germ oil.[4] The present study reveals that both campesterol and Δ^7-stigmastenol are components of rye germ oil, and that they can be isolated by analogous methods, that rye germ oil is almost as rich in campesterol as is wheat germ oil, and is a comparable source of Δ^7-stigmastenol.

Experimental[5]

Preparation of Crude Sterols.—Approximately 45 kg. of thoroughly cleaned and dried rye germs[6] was extracted with petroleum ether (60–68°), giving an orange-brown oil which was saponified under nitrogen and in the presence of pyrogallol with alcoholic KOH solution. The resulting soap, dissolved in the least amount of warm water, on extraction with ethyl ether yielded the unsaponifiable matter, or 8.4% of the oil. It was dissolved in the minimum amount of boiling petroleum ether, cooled to room temperature and stored at 3° for two days. The crude sterols were washed (after recovery) with ice-cold petroleum ether until colorless.

Isolation of Campesterol.—This sterol was recovered from the mixture *via* its acetate.[2] Some 45 g. of crude sterols was acetylated with acetic anhydride and crystallized twice from ethanol–benzene, giving 44 g. of acetate, m.p. 139–140°. A mixture of this acetate, 380 ml. of ether, 530 ml. of acetic acid and 9.3 ml. of bromine was allowed to react overnight. The insoluble material was filtered off, and the soluble portion was debrominated and saponified to give 20 g. of a sterol mixture from which campesterol was crystallized.[2] The mixture was crystallized twice from petroleum ether (60–68°) and then nineteen times from acetone, when a product with constant melting point resulted. In this manner 450 mg. of sterol was obtained, m.p. 157–158°, $[\alpha]^{24}D$ −33.8° (23.7 mg. in 2 ml. of chloroform), a yield of about 1%.

Anal. Calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.10. Found: C, 83.74; H, 12.32.

Derivatives.—The acetate crystallized from ethanol in plates, m.p. 139–140°, $[\alpha]^{24}D$ −37.0° (19.3 mg. in 2 ml. of chloroform).

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.11; H, 11.47.

The benzoate crystallized from methanol–benzene in flat needles, m.p. 158–160°, $[\alpha]^{24}D$ −14.0° (16.0 mg. in 2 ml. of chloroform).

Anal. Calcd. for $C_{35}H_{52}O_2$: C, 83.28; H, 10.39. Found: C, 83.17; H, 10.40.

Reduction of the Acetate.—When 23 mg. of the acetate was hydrogenated with Adams catalyst in glacial acetic acid, the theoretical amount of hydrogen (1.17 ml.) for one double bond was taken up in 20 minutes. The reduced acetate crystallized from methanol–benzene in plates, m.p. 143–144°, $[\alpha]^{25}D$ +18.0° (14 mg. in 2 ml. of chloroform). The infrared spectra of campesterol and its reduced acetate were almost identical with those of β-sitosterol and β-sitostanyl acetate.

Titration with Perbenzoic Acid.—On standing at −5° for 5 days in an excess of perbenzoic acid 14 mg. of campesteryl acetate took up 0.585 mg. of oxygen, corresponding to 1.01 atoms of oxygen per mole of acetate.

Liebermann–Burchard Reaction.—The sterol gave a Liebermann–Burchard reaction similar to that of cholesterol and β-sitosterol.[7] The reduced sterol acetate gave no reaction. A slight variation of the Moore and Baumann modification[8] was used to determine the response of campesterol to this reagent. A 2-ml. aliquot containing 1 mg. of sterol was taken for analysis, to which were added 2 ml. of acetic acid, and then 8 ml. of the 20:1 mixture of acetic anhydride–sulfuric acid. The color was read at intervals using a Coleman Jr. Spectrophotometer with the 620 mμ setting. The sterol reacted at a rate characteristic of the Δ^5-sterols.

Isolation of Δ^7-Stigmastenyl Azoate.—The azoyl esters of the crude sterols were chromatographed according to the procedure used by others for the isolation of Δ^7-stigmastenol from wheat.[4] In a typical case the upper zone (4–6%) was separated from the middle zone (8–10%) by 2 cm. and the middle zone from the lower (82–84%) by 1 cm.

Δ^7-Stigmastenyl Azoate.—The middle zone ester (972 mg.), m.p. 196°, was crystallized from an ethanol–benzene mixture (2:1) until a product with constant melting point was obtained. Five crystallizations gave 520 mg. of ester, m.p. 213–214°.[4]

Δ^7-Stigmastenol and Derivatives.—Hydrolysis of the ester gave 300 mg. of sterol as long needles, m.p. 144–145°, $[\alpha]^{24}D$ +7.9° (13 mg. in 2 ml. of chloroform), after two crystallizations from methanol. The ultraviolet spectrum indicated the presence of 0.6% of a $\Delta^{5,7}$-sterol, but no maxima occurred at the normal concentration used to detect a conjugated system.

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.64; H, 12.05.

The acetate crystallized from ethanol in plates, m.p. 156–157°, $[\alpha]^{24}D$ +6.7° (19.2 mg. in 2 ml. of chloroform).

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.58; H, 11.48. Found: C, 81.48; H, 11.33.

The benzoate crystallized from acetone in plates, m.p. 180–181°, $[\alpha]^{24}D$ +12.0° (17.5 mg. in 2 ml. of chloroform).

Hydrogenation.—When 22.2 mg. of the above acetate was hydrogenated with 20 mg. of Adams catalyst in glacial acetic acid, there was no uptake of hydrogen. The isomerization product crystallized in plates from methanol, m.p. 115°, undepressed by pure $\Delta^{8(14)}$-stigmastenyl acetate.

Titration with Perbenzoic Acid.—On standing at −5° for 5 days, 34 mg. of the acetate consumed 2.40 mg. of oxygen; the theoretical amount for Δ^7-stigmastenyl acetate under these conditions is 2.38 mg.[9]

Additional Properties.—The modified Liebermann–Burchard response of the sterol was characteristic of Δ^7-sterols.[4,8] The maximal millimolar L value was of the chromophore 1920, in good agreement with that reported by Idler, Kandutsch and Baumann.[4] That of the isomerized product, Δ^7-stigmastenyl acetate, was 510, in harmony with their findings. The infrared spectrum of the sterol was identical with that of Δ^7-stigmastenol isolated from wheat, and the spectrum of the isomerized acetate was identical with that of known $\Delta^{8(14)}$-stigmastenyl acetate.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN

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(5) All melting points were determined with Anschütz thermometers totally immersed. Solutions for measurement of specific rotations were made in 2 ml. of chloroform. A Rudolph and Sons Universal high precision polarimeter was used.

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