mole) of antimony pentachloride (5 mole %) were heated at 120° for 24 hours in a stainless steel bomb. The reaction product was rectified through a low temperature Podbielniak column yielding 0.84 g. (0.0069 mole) of dichlorodifluoromethane, 21.5 g. of trichlorofluoromethane and 1.2 g. (0.0078 mole) of carbon tetrachloride.

Disproportionation of *sym*-Tetrachlorodifluoroethane.—A solution of 5.7 g. (0.0191 mole) of antimony pentachloride in 77.5 g. (0.381 mole) of *sym*-tetrachlorodifluoroethane was refluxed as in the catalyst evaluation experiments. The boiling point at the still-head, 91.6°, was unchanged after 10 hours.

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The Separation of D-Glucose and D-Fructose from Invert Sugar or Sucrose[1]

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Cation exchange resins bearing the sulfonic acid group have been employed as catalysts for the condensation reactions of sugars with alcohols to give glycosides.[2,3] Resins of this type have also been used for the hydrolysis of disaccharides, starch and methylated polysaccharides.[3] The hydrolysis of sucrose by this means has been shown to proceed rapidly and quantitatively and the D-fructose may be isolated *via* calcium fructosate.[4]

Condensation reactions between compounds containing the carbonyl group and sugars or sugar derivatives such as glycosides and sugar alcohols have also been catalyzed by cation exchange resins.[2,3] In particular, D-fructose was found to give a high yield of 1,2-4,5-diisopropylidene-D-fructopyranose.[2] Under similar conditions D-glucose does not condense to any large extent with acetone.

A method using cation exchange resins is described herein for the separation of D-glucose from D-fructose. This has been achieved by using a combination of the aforementioned techniques. Thus, when a suspension of finely divided sucrose and a cation exchange resin in acetone containing a small amount (5%) of water is stirred at room temperature, simultaneous hydrolysis of the sucrose and condensation of the derived fructose with acetone take place with the formation of a clear solution. This stage is reached in about 18 hours and soon thereafter crystalline D-glucose begins to separate; eventually a nearly quantitative yield of D-glucose is produced. Invert sugar or a mixture of equal parts of D-glucose and D-fructose can also serve as starting materials. The supernatant solution contains principally the diisopropylidene derivative of fructose and following the removal of excess solvent this compound may be obtained in the crystalline form either directly from the sirup or after purification by solvent extraction. Crystalline D-fructose may then be regenerated by acid hydrolysis either from the crude sirupy diisopropylidene derivative or from the purified crystalline substance.

The success of the method outlined herein depends upon the presence of an empirically determined ratio of water to acetone in order to obtain crystalline D-glucose.

Experimental

Preparation of Resin.—The cation exchange resin, "Amberlite 1R-120,"[5] was regenerated in the usual manner with *N* hydrochloric acid, washed thoroughly with distilled water by decantation to remove all traces of mineral acid and finally washed several times with absolute alcohol and dried at room temperature *in vacuo*. No attempt was made to evaluate the effect of small amounts of mineral acid upon the reactions described herein.[3] In all cases recorded in this paper no mineral acid was detected in the reaction mixtures after removal of the resin.

Separation of D-Glucose from Invert Sugar.—A solution of sucrose (20 g.) in 0.1 *N* sulfuric acid (100 ml.) was heated for 1 hour at 90° on a water-bath. The solution was neutralized while hot with barium carbonate, treated with a little charcoal to facilitate removal of barium sulfate and filtered. Distillation of the solvent *in vacuo* gave the invert sugar as a colorless sirup which was dissolved in water (20 ml.) and shaken at room temperature with acetone (400 ml.) in the presence of Amberlite 1R-120 cation exchange resin (20 g.). After 1 day D-glucose had commenced to separate and crystallization appeared to be complete after 4 days. After 7 days the glucose and resin were filtered off, washed with acetone and dried in air (yield 31.5 g., D-glucose plus resin or 11.4 g. of D-glucose). It is apparent that the yield of D-glucose is nearly quantitative.

Separation of D-Glucose from Sucrose.—Sucrose (50 g.) and Amberlite 1R-120 cation exchange resin (50 g.) were suspended in a mixture of acetone (1000 ml.) and water (60 ml.) and the mixture gently stirred at room temperature. The following changes in the appearance of the reaction mixture were noted: after 14 hours, all the crystalline sucrose had dissolved and the solution was clear; after 24 hours, crystalline-D-glucose appeared and after 72 hours, crystallization appeared to be complete. The reaction mixture was filtered and the D-glucose and resin washed thoroughly with acetone and dried at room temperature. The total weight of D-glucose and resin amounted to 75.6 g. corresponding to a weight of D-glucose of 25.6 g. Without recrystallization the D-glucose so obtained showed $[\alpha]^{22}D$ +44.1° equilibrium value in water (*c* 4.3) and m.p. 130–146°. After one recrystallization the D-glucose showed $[\alpha]^{22}D$ +51.3° equilibrium value in water (*c* 1.0) and m.p. 143–146.

Attempts to duplicate the experiments in the absence of resin met with no success. Invert sugar will not crystallize from aqueous acetone in the absence of the resin treatment nor will sucrose or invert sugar afford crystalline D-glucose when mineral acid is substituted for the resin.

Isolation of 1,2-4,5-Diisopropylidene-D-fructopyranose.—The sirupy solutions derived from the reactions outlined above yielded α-diisopropylidene-D-fructose directly; however, separation of the crystalline derivative from such a sirup is difficult and unnecessary for the regeneration of D-fructose. If the crystalline α-diisopropylidene-D-fructose is required it may be extracted from the sirupy product with benzene, m.p. and mixed m.p. 117–118° (after recrystallization from light petroleum ether).

Regeneration of D-Fructose.—The sirup (22.8 g.), derived from the acetone supernatant solution following the separation of D-glucose from 50 g. of sucrose or invert sugar as described above, was hydrolyzed directly with 0.01 *N* sulfuric acid (220 ml.) at 90°. The solution showed the following change in rotation during hydrolysis: −2.42°, initial value (1-dm. tube); −4.58°, 6 hours; −4.87°, 12 hours (constant value). The hydrolysate was neutralized by passing it over an anion exchange resin (Duolite A-4)[6] and concentrated *in vacuo* to a sirup. This sirup showed upon paper chromatographic examination the presence of a

(1) Paper No. 3132, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul. This paper will form part of a thesis to be submitted by D. S. to the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D.

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(6) Obtained from the Chemical Process Company, Redwood City, Calif.

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.

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

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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.

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(6) Acknowledgment is made to Frank H. Blodgett, Inc., Janesville, Wisconsin, who furnished the material used in this study.

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