[CONTRIBUTION FROM DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION AND THE U. S. REGIONAL SOYBEAN INDUSTRIAL PRODUCTS LABORATORY[1]]

Sterol Glucosides from Cottonseed Oil

BY M. H. THORNTON, H. R. KRAYBILL AND F. K. BROOME

In 1926 Power and Chesnut[2] described the isolation of a phytosterolin from the cotton plant.

This phytosterolin was isolated from the chloroform extract of a resin obtained from the cotton plant by alcohol extraction. After decolorizing with charcoal, the substance was obtained as a white powder, which melted at 218–223° and formed an acetate which melted at 165–166°. Upon hydrolysis, the phytosterolin yielded a substance which reduced Fehling solution, presumably a sugar, and a sterol which melted at 135°. The sugar was not identified.

In a previous publication[3] a method for isolating sterol glucosides from soybean oil by means of an adsorbent[4] was described.

When this method was applied to crude cottonseed oil, this oil was also found to contain some sterol glucosides. In order to compare these compounds with those obtained from soybean oil, a small quantity was isolated from expressed cottonseed oil and its properties were investigated.

Experimental

Isolation of the Glucosides.—The glucosides were isolated by treatment of the crude cottonseed oil with an aluminum silicate adsorbent and subsequent extraction of the adsorbed material with acetone in the same manner as that previously described for obtaining sterol glucosides from soybean oil.

Approximately 36 kg. of crude expressed cottonseed oil was passed through a column containing about 1 kg. of the adsorbent. From the acetone extract of the adsorbed material about 3 g. of sterol glucosides was obtained as a dark powder. This powder gave a positive Liebermann–Burchard test. Since the dark color persisted after four recrystallizations from amyl alcohol, the powder was acetylated by dissolving it in hot pyridine and refluxing for one hour with acetic anhydride. The solution was then evaporated to dryness under reduced pressure and the residue was recrystallized four times from 95% ethanol. The crystals of the acetate were snow-white. The glucosides were regenerated by hydrolysis of this acetate and the recovered glucosides were washed and dried *in vacuo* at 100°. The product darkened at 240° and melted with decomposition at 248–250°.

Analysis of the Glucosides. Calcd. for $C_{35}H_{60}O_6$: C, 72.85; H, 10.49. Found: C, 71.57, 71.78; H, 10.60, 10.61.

Acetylation of the Glucosides.—A 1-g. sample of the regenerated glucosides was acetylated by boiling for one hour with 20 ml. of pyridine and 10 ml. of acetic anhydride. The acetate was recovered and recrystallized four times from 95% ethanol. The yield was about 0.8 g.; m. p. 164–165°; $[\alpha]^{20}D$ in chloroform $-40.5°$ ($c = 2, l = 2$).

Analysis of the Acetate. Calcd. for $C_{43}H_{68}O_{10}$: C, 69.29; H, 9.20. Found: C, 69.58, 69.61; H, 9.41, 9.49.

The acetyl groups were determined by hydrolysis of the acetate in alcoholic solution with 0.1 *N* sodium hydroxide and titration of the excess alkali with 0.1 *N* sulfuric acid. Calcd. for $C_{43}H_{68}O_{10}$: CH_3CO, 23.13. Found: CH_3CO, 23.0, 23.15.

Hydrolysis of the Glucosides.—The glucosides were hydrolyzed by the method used for the hydrolysis of sterol glucosides from soybean oil.[3] A 1.44-g. sample was refluxed for ten hours with 100 ml. of absolute ethanol and 2 ml. of concentrated hydrochloric acid and the alcohol partially removed under vacuum. Water was added and the sterols were filtered off. The aqueous solution was then refluxed for six hours in order to hydrolyze the ethyl glucoside. At the end of this period analysis revealed the presence of 343 mg. of sugar calculated as glucose. The solution was neutralized with silver carbonate, a small amount of charcoal added, and the silver chloride removed by filtration. The clear filtrate was evaporated to a thick sirup under reduced pressure and the benzimidazole was prepared by the method of Moore and Link.[5] The benzimidazole melted at 213° and recrystallization of it did not change this value. The benzimidazole from an authentic sample of *d*-glucose melted at 214° and a mixture of the two melted at 213°.

The picrate of the benzimidazole was prepared and, after recrystallization, melted at 207°, alone or when mixed with a sample of known glucobenzimidazole picrate. Therefore, it may be concluded that the sugar derived from the hydrolysis of the sterol glucosides of cottonseed oil is *d*-glucose.

The sterols, which were obtained by hydrolysis of the glucoside, were recrystallized three times from 95% ethanol: m. p. 128–130°; $[\alpha]^{20}D$ in chloroform -25.07 ($l = 2$, $c = 1.4$).

A sample of 0.35 g. of these sterols was acetylated by boiling for two hours with 15 ml. of acetic anhydride. On cooling, the acetylated product crystallized and was removed by filtration. The yield was 0.25 g. This acetate was recrystallized three times from 95% ethanol and pure white crystals were obtained: m. p. 112–114°; $[\alpha]^{20}D$ $-23.50°$ ($l = 2$, $c = 0.8$).

(1) The U. S. Regional Soybean Industrial Products Laboratory is a coöperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the United States Department of Agriculture and by the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

(2) Power and Chesnut, THIS JOURNAL, **48**, 2721 (1926).

(3) Thornton, Kraybill, and Mitchell, *ibid.*, **62**, 2006 (1940).

(4) Kraybill, Brewer, and Thornton, U. S. Patent 2,174,177 (1939).

(5) Moore and Link, *J. Biol. Chem.*, **133**, 293 (1940).

Discussion

The mixture of sterol glucosides isolated from cottonseed oil resembles that isolated from soybean oil very closely. The melting point of the glucoside mixture from soybean oil is higher than that of the mixture from cottonseed oil. Both glucoside mixtures formed tetraacetates with practically the same melting points but with quite different rotatory powers. Since both glucoside mixtures contain *d*-glucose, it is apparent that these differences must be due to the sterols. The sterols from the soybean glucoside mixture were shown to contain about 25% of stigmasterol,[3] while a number of investigators have reported that this sterol was either entirely absent or present in only small amounts in the free sterols of cottonseed oil.

The sterol glucoside mixture from cottonseed oil differs somewhat from that isolated from the cotton plant by Power and Chesnut. The differences lie chiefly in the higher melting point of the glucoside mixture from the oil and in the lower melting point of the sterols obtained by the hydrolysis of this mixture.

Summary

Mixed sterol glucosides were isolated from cottonseed oil by treatment of the oil with an adsorbent and subsequent extraction of the adsorbed material with acetone.

The sugar obtained by acid hydrolysis of the glucosides was identified as *d*-glucose.

The mixed sterol glucosides isolated from cottonseed oil differ in some respects from those isolated from the cotton plant by Power and Chesnut. These differences are apparent in the higher melting point (248–250°) of the glucosides from the oil and the lower melting point (128–130°) of the sterols which these glucosides yield on hydrolysis.

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Heats of Organic Reactions. XI. The Denaturation of Pepsin by Alkali

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In the investigation of the heat of denaturation of methemoglobin by alkali[1] several experimental difficulties were encountered. A comparison of the heats of reaction of native and denatured methemoglobin with alkali could only be made by an extrapolation. Rapid denaturation of methemoglobin occurred at *p*H 11 or greater; at this *p*H considerable disintegration of the protein molecule into fragments is possible. It therefore seemed advisable to choose for further work a protein denaturable at a lower *p*H than methemoglobin. Pepsin is rapidly and completely denatured at *p*H 8.0,[2] and has the further advantage of being soluble in the native and in the denatured condition, in the concentrations used in our experiments, at *p*H 4.3, thus permitting a direct comparison of heats of reaction of native and denatured protein with alkali.

Experimental Procedure

Calorimetric Technique.—The calorimeter used in these experiments has been described in a previous paper of this series.[1] Since performing the experiments described there, the apparatus has been slightly altered to eliminate one source of uncertainty. Addition of alkali changes the viscosity of protein solutions markedly and thus alters the heat of stirring. It is therefore impossible to establish a generally valid relation between the thermal head of the calorimeter and the rate of its temperature change. The result is an uncertainty in the heat of any reaction which does not occur practically instantaneously. A pneumatically operated clutch was installed on the stirrer shaft of the working calorimeter, operated from the outside of the air thermostat surrounding the water thermostat bath. The stirring was repeatedly interrupted for short measured intervals of time (usually five minutes) during the course of a reaction, and the rates of temperature change with and without stirring calculated from systematic readings of the e. m. f.'s of the main and adiabatic thermel. In absence of stirring (and chemical reactions) the rate of temperature change of the calorimeter has been found to be solely determined by its thermal head above the thermostat, as read on the adiabatic thermel. Thus the knowledge of the rates of temperature change with and without stirring in presence of chemical reactions is sufficient to separate for the purpose of calculation the three contributing factors: heat conduction to the outside, heat of stirring and the heat of chemical reaction.

The protein solutions were made up to 840 g. and poured into the outer compartment of the calorimeter. The proper quantity of alkali was diluted to 62 cc. and placed

(1) Conn, Kistiakowsky and Roberts, THIS JOURNAL, **62**, 1895 (1940).

(2) Philpot, *Biochem. J.*, **29**, 2458 (1935), has found that pepsin brought to *p*H 11 remained homogeneous in the ultracentrifuge.