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

Sterol Glucosides from Expressed Soybean Oil[2,3]

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Sterol glucosides have been obtained from many plants by various methods of extraction. Jantzen and Gohdes[4] isolated sitosteryl-*d*-glucoside by extracting soybeans with benzine and saponifying the oil obtained. The unsaponifiable matter was extracted with ethyl ether and the fatty acids were liberated from the soap with hydrochloric acid. When these fatty acids were shaken with ethyl ether, the insoluble glucoside collected at the water–ethyl ether interface and was separated by filtration. These investigators also obtained the same glucoside from the phosphatides, which are especially rich in this material.

According to Jantzen and Gohdes, crude solvent extracted soybean oil was found to contain 0.03% of sterol glucoside, whereas phosphatides prepared by washing the oil with water contain up to 3.0%, and the phosphatide-free oil contains none at all. Glucose was identified in the form of the diphenylhydrazone after hydrolysis of the glucoside. The sterol was identified as sitosterol but the melting point, 138.4 to 138.9°, is similar to that of the sterols isolated from soybean oil, which are known to be mixtures of sitosterols and stigmasterol.

Kondo and Mori[5] obtained sitosteryl-*d*-glucoside from soybeans by extracting the residue from successive extractions with acetone, methanol, and ethyl ether with hot 80% methanol.

In the present investigation the mixed sterol glucosides have been prepared in large quantities from crude expeller soybean oil by treatment of the oil with an adsorbent and subsequent extraction of the glucosides from the adsorbent.

(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 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) From a thesis presented to the Faculty of Purdue University by M. H. Thornton in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1937.

(3) Presented before the Division of Agricultural and Food Chemistry at the 98th meeting of the American Chemical Society, Boston, Massachusetts, September 11, 1939.

(4) Jantzen and Gohdes, *Biochem. Z.*, **272**, 166 (1934).

(5) Kondo and Mori, *J. Chem. Soc. Japan*, **57**, 1128 (1936); *C. A.*, **31**, 733 (1937).

Experimental

Preparation and Purification of the Glucosides.—A synthetic aluminum silicate adsorbent,[6] which has a great affinity for the phosphatides and materials associated with them in vegetable oils, was prepared. When crude soybean oil is treated with this material, complete removal of the phosphatides is effected. Many of the associated materials such as sterols, glucosides, and pigments are also either partially or completely removed from the oil.

Approximately 4550 kg. of crude expeller soybean oil was treated with 30 kg. of the adsorbent. After separating the treated oil from the adsorbent as completely as possible by mechanical means, the adsorbent was extracted with acetone. The residue left after evaporation of the acetone was an oil which, on cooling to room temperature, deposited a white flocculent precipitate. This material was collected by filtration and washed with acetone and ether. The separated solid gave a positive Liebermann–Burchard reaction. After it was recrystallized three to five times from *n*-amyl alcohol it darkened at 250–255° and melted with decomposition at 267–270°. It was acetylated by dissolving it in boiling pyridine and refluxing for one hour with acetic anhydride. This solution was evaporated to dryness under reduced pressure and the acetate recrystallized four times from 95% ethanol. The original glucosides were regenerated by saponification of the acetyl derivative. After saponification the glucosides were washed with water, ethanol, and ethyl ether and dried at 100° *in vacuo*. Approximately 235 g. of the glucosides was obtained.

Analysis of the glucosides. Calcd. for $C_{35}H_{60}O_6$: C, 72.85; H, 10.49. Found: C, 71.93, 72.12; H, 10.47, 10.55.

Acetylation of the Glucosides.—The purified glucosides were acetylated by boiling 2 g. of the product for one hour with 25 ml. of pyridine and 10 ml. of acetic anhydride. The solution was evaporated to dryness under reduced pressure and the residue recrystallized four times from ethanol. Approximately 1.8 g. of glistening crystals was recovered: m. p. 165–166°, $[\alpha]^{20}D$ in chloroform −24.5° (c = 2.0, l = 2). Further recrystallization did not change these constants.

A determination of the acetyl groups was made by saponification 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, 23.2, 23.5.

Crystallographic Optical Properties of the Glucoside Tetraacetate.[7]—The tetraacetyl glucosides were recrystallized from ethanol and dried in high vacuum at 70°.

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

(7) Optical examination made by Dr. E. D. Walter of this Laboratory.

Under the microscope in ordinary light the crystals have the appearance of thin laths. In parallel polarized light (crossed nicols) the extinction is parallel and the sign of elongation is positive. The birefringence is weak. In convergent polarized light (crossed nicols) the substance is biaxial. An occasional narrow face is found on which no extinction occurs on rotating the stage. These occasionally observed narrow faces show the emergence of an optic axis interference figure. The optic sign as determined on these figures is negative. On many of the broader faces an obtuse bisectrix emerges. The refractive indices are: $\alpha = 1.512$ (shown crosswise), $\beta = 1.522$ (on rods showing emergence of an optic axis), both ± 0.003, γ = not determined. The α index is the most commonly occurring and is always shown across the broad faces of the crystals when their long dimension lies perpendicular to the vibration plane of the lower nicol.

Regeneration of the Glucosides.—A 2-g. sample of the purified glucoside tetraacetate was refluxed for one hour with 100 ml. of ethanol containing 0.5 g. of sodium hydroxide. The solution was cooled and the precipitated glucosides recovered by filtration, washed with water, ethanol, and ethyl ether and dried at 100° *in vacuo*. When heated in a melting-point tube, the regenerated product darkened at 250–255° and melted with decomposition at 267–270°.

Hydrolysis of the Glucosides.—The glucosides were hydrolyzed with a mixture of amyl alcohol, 15% hydrochloric acid, and ethanol according to the directions of Power and Salway.[8] The reaction mixture was carefully evaporated to dryness under reduced pressure and the sugar and sterols separated by extraction with water and ethyl ether. This method of hydrolysis has several disadvantages, namely, the high temperature necessary during the boiling and evaporation of the amyl alcohol solution tends to cause decomposition of the sugar and it is quite difficult to remove the hydrochloric acid from the aqueous solution by evaporation. Quantitative determinations on hydrolyzates obtained in this way showed the presence of only a small fraction of the theoretical quantity of sugar. In order to identify the sugar a portion of this hydrolyzate was subjected to the Orcin test for pentoses and Pinoff's test for ketoses as described by van der Haar.[9] Similar tests were made for comparison on samples of known sugars. The Orcin test for both pentoses and methyl pentoses was negative. Pinoff's test for ketoses was also negative. These tests indicate that a pentose is not present and also that the sugar is not a ketose.

Further work led to an improvement in the method of hydrolysis whereby positive identification of the sugar was possible. In the latter method 7 g. of sterol glucosides was refluxed for twelve hours with 300 ml. of absolute ethanol and 2 ml. of concentrated sulfuric acid, after which the ethanol was removed by evaporation under reduced pressure. No charring occurred. This may have been due to the formation of ethyl hydrogen sulfate from the sulfuric acid and ethanol. The recovered sterols were dissolved in ethyl ether and the ethereal solution extracted with small volumes of water. The aqueous solution showed only a slight reducing power and apparently contained the sugar in the form of the ethyl glucoside. It was refluxed for five hours and then was found to contain slightly less than the theoretical amount of free sugar calculated as glucose. The sulfuric acid was removed with barium carbonate and the solution decolorized with a small amount of activated vegetable charcoal. Hydrochloric acid can also be used for the hydrolysis since it can easily be removed by neutralization with silver carbonate. The clarified solution was concentrated to 4 ml. under reduced pressure and the benzimidazole prepared according to the method of Moore and Link.[10]

The benzimidazole prepared from an authentic sample of *d*-glucose melted with decomposition at 214.5°. The benzimidazole prepared from the unknown sugar melted with decomposition at 215° and a mixture of it with *d*-glucobenzimidazole melted with decomposition at 214.5°. A solution in 5% citric acid had a specific rotation at 25° of +10.2° ($l = 2$, $c = 1.6$). Moore and Link give the following values for *d*-glucobenzimidazole: m. p. 215°, $[\alpha]^{25}D$ +9.6°. Therefore, it may be concluded that the sugar derived by hydrolysis of the sterol glucosides from soybean oil is *d*-glucose. This observation confirms the previous work of Jantzen and Gohdes.

The mixed sterols recovered from the hydrolyzate gave a positive Liebermann-Burchard reaction and after recrystallization from ethanol melted at 133.5–135° and had a specific rotation of −36.82° (chloroform) at a temperature of 20° ($c = 2.0$, $l = 2$).

A 10-g. portion of these sterols was acetylated by refluxing for one hour with 100 ml. of acetic anhydride. The solution was cooled and 9.9 g. of the crystalline acetyl derivative recovered by filtration. After purification by recrystallization from ethanol it melted at 133–134° and had a specific rotation of −45.94° (chloroform) at 20° ($c = 1.6$, $l = 2$).

Stigmasteryl acetate tetrabromide was prepared by the method of Windaus and Hauth.[11] A portion of sterol acetate weighing 5.3 g. was dissolved in 53 ml. of ethyl ether and 66 ml. of a 5% solution of bromine in glacial acetic acid was added slowly with shaking at room temperature. After cooling at about 5° for two hours, 2.24 g. of crystals, m. p. 190°, was recovered. This material was then recrystallized three times from a mixture of benzene and methanol. The purified product melted at 198°. A 2-g. sample of the purified material was dissolved in a mixture of 50 ml. of glacial acetic acid and 50 ml. of ethanol and debrominated by refluxing for two hours with 2 g. of zinc dust. The solution was filtered while hot to remove the zinc and the solid material thrown out of solution by the addition of water. After filtration the recovered solid, recrystallized three times from ethanol, melted at 140–141°. This material was saponified by refluxing a 1-g. portion for two hours with 50 ml. of ethanol and 5 ml. of 50% potassium hydroxide. Water was then added to the warm solution until it became turbid and the crystalline material was filtered off and washed with water. After three recrystallizations from 95% ethanol, it melted at 169° and had a specific rotation in chloroform

(8) Power and Salway, *J. Chem. Soc.*, **103**, 399 (1913).

(9) "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und and Aldehydesäuren," A. W. van der Haar, Gebrüder Borntraeger, Berlin, 1920.

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

(11) Windaus and Hauth, *Ber.*, **39**, 4378 (1906).

at 20° of −51.82° (c = 2.103, l = 2). Sandqvist and Gorton[12] give the following values for stigmasterol isolated from soybean sterols: m. p. 169–170°; $[\alpha]_D$ −51.0°.

The stigmasterol content of the mixed sterols, as calculated on the basis of the crude stigmasteryl acetate tetrabromide, is approximately 24% but some loss is experienced during purification so that this amount of stigmasterol is not recovered.

In respect to stigmasterol content, melting point, and specific rotation the glucosidic sterols bear a very close resemblance to the sterols occurring in the uncombined state in soybean oil.

Summary

1. Sterol glucosides occur to an appreciable extent in commercial expeller soybean oil.

2. These glucosides were removed from the oil by adsorption methods and were obtained by acetone extraction of the adsorbed material.

3. The sugar was obtained from the glucosides in almost theoretical yield by first forming the ethyl glucoside and subsequently hydrolyzing the easily soluble ethyl glucoside. The sugar was identified as *d*-glucose.

4. The sterols obtained by hydrolysis of the glucosides are very similar to the uncombined sterols of the oil and consist of a mixture of sterols in which stigmasterol occurs to the extent of approximately 24%.

(12) Sandqvist and Gorton, *Ber.*, **63**, 1935 (1930).

LAFAYETTE, INDIANA RECEIVED APRIL 15, 1940

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

The Crystal Structure of Rhombohedral Acetamide

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I. Introduction

The purpose of this research was to investigate the properties of the N–H–O bridge. Some such bridging must exist in crystalline acetamide, for, while it has the approximate molecular weight and is isoelectronic with acetone and 1,1-dimethylethylene, its melting point and boiling point are approximately 100° higher, thus indicating much stronger intermolecular forces.

We should expect two N–H–O bridges from each N in acetamide. One such bridge could be formed intramolecularly, but this is unlikely. Such a bridge should be weaker than a corresponding O–H–O bridge in formic acid; the structure of formic acid vapor[1a] and molecular weight determinations indicate that formic acid is a dimer and that no intramolecular bridging occurs. Hence, we expect no intramolecular bridge in acetamide. If bridges are formed to other molecules there is no reason to suppose that the direction of the bridge is not the direction of the bond from one of the terminal atoms in the bridge to hydrogen. Consequently, locating the N–H–O bridges also partially locates the H atoms. The determination of the crystal structure of acetamide will thus furnish a great deal of information about the position of the bridging hydrogen atoms, although they have no appreciable effect on the diffraction of X-rays.

The crystal structure of acetamide as determined by this investigation leads us to the conclusions (a) that the lengths of the N–H–O bridges are 2.86 ± 0.05 Å.; (b) the molecule of acetamide is planar; (c) the molecule exists in the keto form; and (d) the N–H bonds lie in the plane of the molecule.

II. Determination of the Unit Cell and Space Group.—Crystals of acetamide in the form of transparent, trigonal prisms were obtained by slowly cooling ethyl acetate saturated with C. P. acetamide at 50°. The prism faces on the crystals were well developed but rapidly disappeared upon exposure to air due to the extremely deliquescent nature of the compound. The crystal was mounted on a small glass rod by means of cellophane tape and this was placed in a thin-walled bulb blown from a 2-mm. Pyrex tube. The entire operation of mounting the crystal from the time it was removed from the mother liquid until it was mounted in its final position was done in a moisture-free atmosphere.

Three sets of oscillation photographs were taken: a 30° oscillation about the c-axis, a set of four 30° oscillations about the a-axis of the hexagonal cell, and a set of four 30° oscillation photographs about the [10·0] axis.

Measurements on these photographs gave values of a_0 = 11.44 ± 0.03 Å., and c_0 = 13.49 ± 0.03 Å. for the dimensions of the hexagonal unit

(1) American Can Company Fellow.

(1a) L. Pauling and L. O. Brockway, *Proc. Nat. Acad. Sci.*, **20**, 336 (1934).