are suspended is heated from 80° C. to boiling. Granule size increases greatly and much more folding and wrinkling becomes apparent (Figure 2, *I, J*). Prolonged heating at one temperature produces relatively little change beyond that seen when the temperature is first attained (Figure 2, *K, L*); however, after heating for several days the granules often shrink and become very difficult to break.

Starch in Situ

The appearance of pieces of the grain in which starch occurs is sometimes helpful in identifying the grain. Corn, for example, gives two types of endosperm pieces. In fragments of floury endosperm, the cell walls are rarely visible and the spherical granules of starch appear to be loosely arranged without any particular order (Figure 3). In pieces of horny endosperm, the cell walls are obvious and the polyhedral starch granules appear to be packed in an orderly mosaic. Sorghum endosperm fragments appear similar to those of corn. Fragments of barley, wheat, and rye endosperms look somewhat like the pieces of floury endosperm of corn, but the two sizes of granules are evident, the small spherical granules lying between and often surrounding the large lens-shaped granules.

Appearance of starch in the hull can be used to distinguish sorghum from corn and wheat (Figure 4). Corn pericarp, like wheat pericarp, which forms the major portion of the hull, contains no starch. In contrast, sorghum pericarp includes cells containing starch granules much smaller than those occurring in the endosperm, which give the fragment of sorghum pericarp a somewhat stippled appearance. Large granules may be washed in from endosperm as the material is mounted.

Familiarity with known mixtures of cereal starches will aid the microscopist to recognize similar mixtures in samples of unknown composition. Sometimes it is possible to estimate proportions of the different starches present and so to gain some indication of the percentage composition of the sample.

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WHISKEY CLARITY

Isolation and Identification of a Sterol Glucoside from Whiskey

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A hitherto unrecognized factor in the clarity of whiskey has been identified as sitosterol β-D-glucoside. The glucoside is of plant origin and originates in the finished beverage as a barrel extractive. From 60 pounds of white oak wood, 0.20 gram of the glucoside was isolated.

THE DISTILLER, endeavors to satisfy the consumer and the industry, to produce a visually clear and sparkling product free of any trace of haze or cloudiness. The major cause of turbidity in whiskey is the presence of high molecular weight, free fatty acids and their esters (*1*). These are carried over in the high wine during the distillation of the fermented grain mash and originate from the oils in the grains and from the metabolic products of the yeast.

At least two successful, though empirical, methods have been developed for the removal of these bothersome fats. Either chill filtration or active carbon treatment of whiskey is used today by most distillers to produce a generally satisfactory product.

Some bonded whiskies and more especially the lower proof straight whiskies, even after such treatments, sometimes develop a slight precipitate on storage in the bottle. This paper presents the isolation and the physical and chemical data of this material, and proves the identity of this substance to be sitosterol β-D-glucoside.

Experimental

Several bottles of carbon-treated 86-proof straight whiskey containing the characteristic precipitate were filtered in an effort to determine the nature of this material. The retained matter was washed with cold diethyl ether to determine the presence of any fatty substances. The ether on evaporation yielded no residue, indicating the absence of fatty acids or esters. The residue remaining on the filter paper was then continuously extracted with diethyl ether and after several hours a white floc collected in the lower flask of the Soxhlet apparatus. The floc was filtered from the ether, air dried, and then vacuum dried. Infrared absorption spectra and a melting point of about 282–92° C., with decomposition, was obtained for the compound. It also gave a positive and classical response to the Liebermann-Burchard test and a positive Salkowski-Hesse reaction for sterols. The quantities obtained, however, were insufficient for complete and positive identification.

To obtain a sufficient quantity of material for further investigation, the complete filter cake (diatomaceous earth) and precoat from the filtration of 2500 gallons of 86-proof straight whiskey (chilled to 17° F. and maintained for 48 hours) was airdried for several days on large copper trays. The dried material was then placed in cloth bags and charged in the side extraction chamber of a large modified Soxhlet extractor and continuously extracted with diethyl ether for at least 48 hours. All ether extracts from 15 such extractions were combined, and most of the ether was evaporated, leaving a volume of 800 ml. This solution was filtered, and the residue was washed with 50 ml. of cold ethyl alcohol, which removed most of the coloring matter. The residue, gray in color, consisted largely of siliceous filter aid, not

retained by the cloth bags, in admixture with the desired compound.

The residue was extracted continuously with hot 95% alcohol in a vapor-jacketed Soxhlet extractor for 96 hours. A heavy white floc separated in the lower flask. The flask and contents were cooled to 0° C. and filtered. The precipitate after thorough vacuum drying weighed 4 grams. Five recrystallizations from alcohol (95%) using Norite yielded a pure white microcrystalline compound of needlelike structure, melting at 280° to 292° C. with decomposition. The broad melting range with decomposition is often typical of sugars or sugar containing compounds. The compound gave a positive Molisch test for carbohydrate and a positive Liebermann-Burchard test. This information and infrared absorption spectra showed this compound to be identical with the compound recovered from the bottled whiskey.

The purified compound was found to be sparingly soluble in ethyl alcohol, methanol, benzene, chloroform, and ethyl acetate; insoluble in water, dilute acid, and dilute alkali; and soluble in pyridine and amyl alcohol. The optical rotation of the compound determined in pyridine was $[\alpha]_D^{25°} = -41.6°$. Acetylation of the compound with acetic anhydride in pyridine gave an acetate, melting sharply at 167° C. The acetate, which gave a positive response to the Liebermann-Burchard test, was hydrolyzed with dilute alkali, yielding the original compound which had the same melting point of 280° to 292° C., with decomposition. This indicated that the purity of the original compound could not be appreciably improved by acetate formation and was pure in spite of the melting point range.

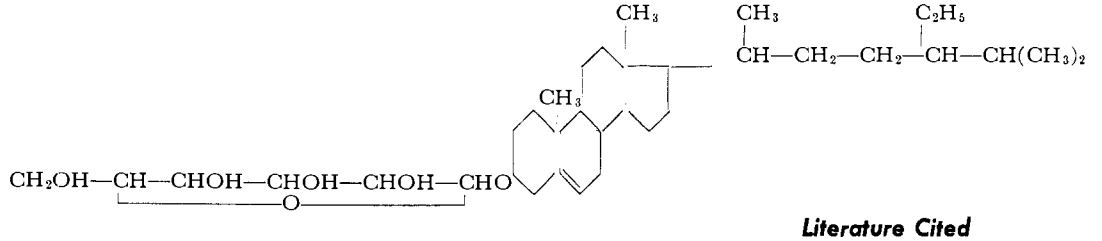
The unknown compound was next hydrolyzed with acid in an attempt to isolate the sterol and sugar components by rupture of the presumed acetal linkage. After several attempts, the method of Wall and coworkers (*4*), with some modification, proved successful. Two hundred milligrams of the unknown compound were dissolved in 50 ml. of ethyl alcohol, to which was added an equal volume of water. The solution was made 4*N* with hydrochloric acid and heated under reflux for 24 hours. At no time during the reaction was all of the compound in solution, but this did not appear to affect the hydrolysis. The flask was cooled to room temperature and its contents were diluted with water and extracted with benzene. The benzene was evaporated and the residue recrystallized from ethyl alcohol, followed by several recrystallizations from ether. The sterol melted at 136° C., the melting point of β-sitosterol. Confirmatory evidence was obtained by preparing the acetate of the sterol which melted at 126–7° C. and is identical to β-sitosterol acetate.

The sugar was isolated from the water-alcohol-acid solution by the method of Cloetta (*2*). The acid was neutralized with silver carbonate, the excess silver removed with hydrogen sulfide, and the resulting clear solution was extracted with chloroform to remove any remaining sitosterol. The aqueous solution was concentrated under reduced pressure and used directly in the chromatographic procedure of Partridge (*3*). The chromatogram showed the presence of only one sugar having an R_f value of 0.39, which is identical to D-glucose.

The data indicated the compound to be sitosterol β-D-glucoside. Complete confirmation was obtained by comparison of the elemental compositions. The table shows this comparison:

	C, %	H, %	O, %	Mole Wt.	M.P., °C.
Unknown compound	72.45	10.70	17.04	565	282–92
Sitosterol β-D-glucoside	72.87	10.48	16.64	576.83	285–92
Unknown compound, acetate	69.37	9.25	21.44	733	167
Sitosterol β-D-glucoside tetraacetate	69.32	9.20	21.47	744.97	166–8

The evidence thus presented proves unequivocally that the compound isolated from carbon-treated bottled whiskey is identical to the compound obtained from chill-filtered whiskey. This compound is sitosterol β-D-glucoside, also known as sitosterolin and phytosterolin, having the following formula:



Isolation of Glucoside from Wood

Sixty pounds of white oak wood was placed in a modified Lloyd extractor and continuously extracted with 10 gallons of 95% ethyl alcohol for 4 weeks. The alcoholic extract was reduced in volume by distillation under reduced pressure to 2 liters. The solution was cooled to near 0° C. and filtered, leaving a large chocolate-colored residue and a dark brown filtrate.

The residue was air dried, placed in a modified Soxhlet extractor, and continuously extracted with diethyl ether for 2 weeks. After that time, the ether was filtered from the precipitated material which proved to be sitosterol β-D-glucoside. Recrystallization from ethyl alcohol yielded a crystalline compound melting at 285-9° C. The infrared spectrum of the compound was identical to sitosterol β-D-glucoside.

A total yield of 0.20 grams of glucoside was obtained, showing the glucoside content of this wood to be approximately 0.0007%.

Discussion

The origin of sitosterol β-D-glucoside in whiskey is extremely interesting from a chemical and biogenetic viewpoint. Of more practical importance, this knowledge may lead to a feasible and economical method for its removal from whiskey. This compound is of plant origin and, therefore, must arise in whiskey by entrainment distillation during the manufacture of the high wine or as a barrel extractive. Isolation of the sterol glucoside from white oak wood shows the latter to be true.

The glucoside according to Whitby (*5*) behaves as an organophilic colloid, swells and disperses on heating in a variety of organic liquids, and forms gels on cooling such dispersions. This may explain some of the difficulties associated with the sporadic presence of this compound in whiskey.

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