carbon, natural caffeine from theobromine 87% of modern carbon, and natural caffeine from tea 100% of modern carbon. These data confirmed that caffeine from tea contained eight modern carbon atoms and caffeine prepared by methylation of theobromine contained the equivalent of seven modern carbon atoms out of a total of eight in the caffeine structure. The synthetic caffeine prepared from urea contained the equivalent of three modern carbon atoms. The presence of this modern carbon was explained after a check with a supplier showed the methyl chloride (used in the synthesis of caffeine) to have been obtained from methanol prepared by the destructive distillation of wood. Another synthetic reference sample contained one modern carbon due to the use of formic acid made from methanol in the synthesis. Hence, the amount of modern carbon in synthetic caffeine can vary, but it never has approached the equivalent of seven or eight modern carbons, since known synthetic processes do not use raw materials totally derived from natural or modern carbon sources. On this basis, an unknown showing less than the equivalent of seven modern carbons by radioactive C^{14} measurement is classified arbitrarily as synthetic material.

Questionable caffeines (Table II) which had been identified by the infrared method as from an uncertain or synthetic source instead of the natural source claimed by the supplier were examined by the radiocarbon method. Samples *A* and *B* produced residue infrared spectra containing bands peculiar to urea and coffee caffeine, but no theobromine bands. This shows that the products were not derived from theobromine. Since a positive identification could not be made, the samples were classified as unidentified. Samples *C* and *D* produced spectra possessing distinct characteristic urea caffeine bands. On this basis, these samples were classified as synthetic.

Results with the radiocarbon method showed that all the samples contained 48% or less of modern carbon and must be classified as synthetic caffeine. These findings confirmed that the samples were not as labelled. The analysis of 11 additional synthetic samples, some from an unknown source, showed an average of 13.2% of modern carbon with a spread ranging from 11 to 15%.

Acknowledgment

Appreciation is expressed to R. B. Woodward, Harvard University, who suggested the use of carbon dating for distinguishing natural from synthetic caffeine. The invaluable assistance of L. A. Hertling and W. J. Detroit, who prepared samples for radiochemical and infrared analyses, and of O. E. Kinast, who obtained the infrared spectral data, is gratefully acknowledged.

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PEANUT FLOUR CONSTITUENTS

Isolation and Identification of a Sterol Glucoside from Peanut Flour

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CONSTITUENTS of the nonoil, alcohol-soluble fraction of the peanut are being studied as a part of a program to relate composition of raw peanuts to the quality of processed peanut products, with particular attention to those present in small quantities. The present report is concerned with β-sitosterol-D-glucoside.

Experimental

In the preparation of the nonoil alcohol-soluble fraction, Spanish peanuts were blanched, freed from skins, sliced, and extracted with commercial hexane (60° to 70° C.) until the oil content of the residue was less than 0.1%. The air-dried flour was exhaustively extracted with 95% ethyl alcohol. The alcohol extract was concentrated by vacuum distillation and subsequently lyophilized to yield a dry product representing 7% of the flour, or 3.5% of the blanched peanut kernel (*B*, Figure 1).

Sufficient 50% aqueous alcohol was added to 300 grams of the dry extract to dissolve it. The resulting solution was treated with powdered neutral lead

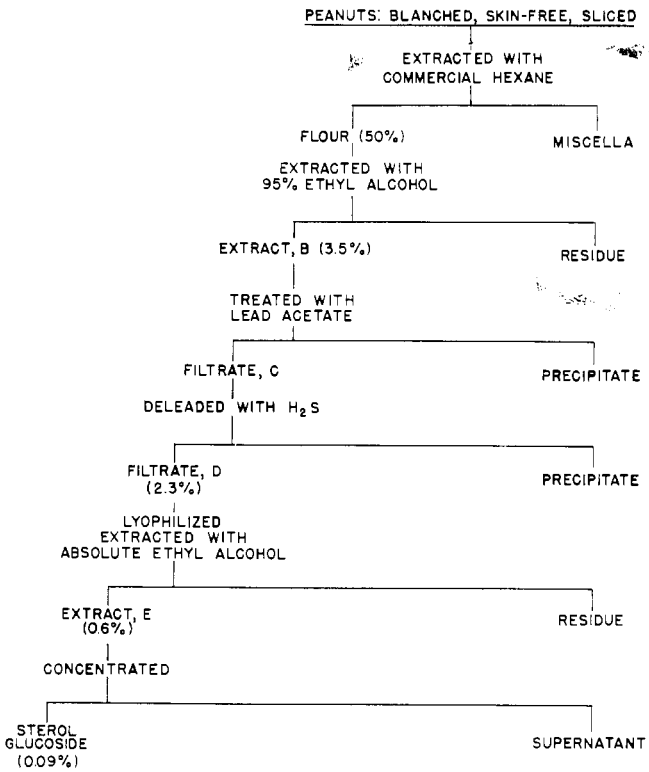

Figure 1. Schematic diagram of isolation of sterol glucoside

β-Sitosterol-D-glucoside was isolated from lipide-free peanut flour and its identity established by elemental analysis, infrared absorption, and glass paper chromatography in comparison with an authentic sample. It represents about 0.09% of the skin-free peanut kernel.

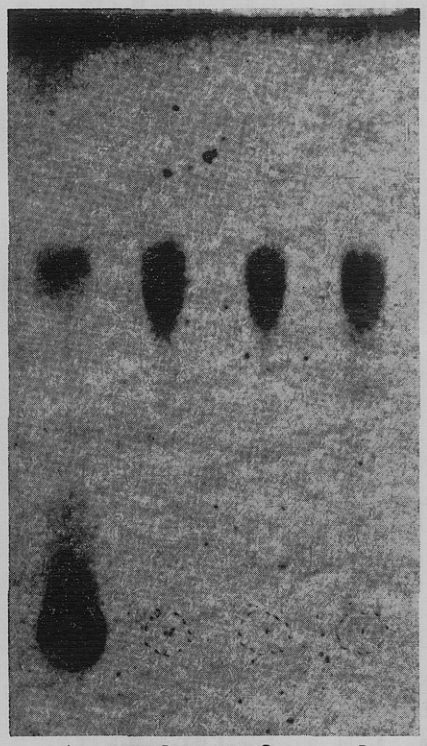

Figure 2. Chromatogram showing separation of β-sitosterol-D-glucoside from solution E on silicic acid–impregnated glass paper

A. Solution E
B. Authentic β-sitosterol-D-glucoside
C. Glucoside separated from solution E by silicic acid column
D. Glucoside separated from solution E by precipitation on concentration and standing

acetate until clarification was complete. The solution was filtered. The filtrate (*C*, Figure 1) was treated with hydrogen sulfide to remove the excess lead, which was removed by filtration. A concentrate of the filtrate (*D*, Figure 1) was lyophilized and yielded 200 grams, which represented 2.3% of the blanched peanut kernel.

This dried material was extracted with absolute alcohol. Approximately 100 ml. of alcohol per gram of dried material were used to ensure complete extraction. This extract (*E*, Figure 1) was concentrated under reduced pressure, care being taken that the temperature of the water bath did not exceed 60° C. A white flocculent precipitate separated from the concentrated solution upon cooling and standing.

This crude precipitate was collected by centrifugation, and washed sucessively with cold ethyl alcohol, diethyl ether, carbon disulfide, and water.

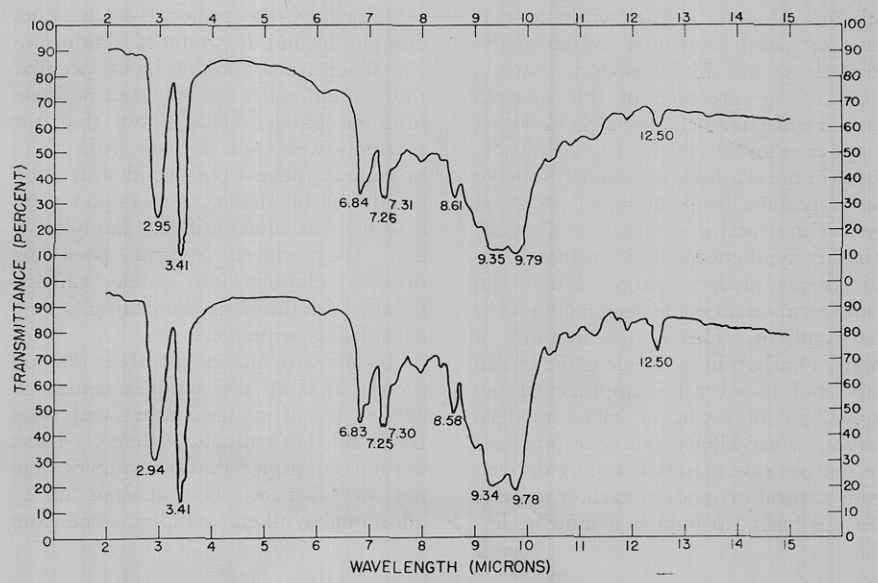

Figure 3. Infrared absorption spectra

A. Glucoside isolated from peanut flour
B. Authentic sample of β-sitosterol-D-glucoside

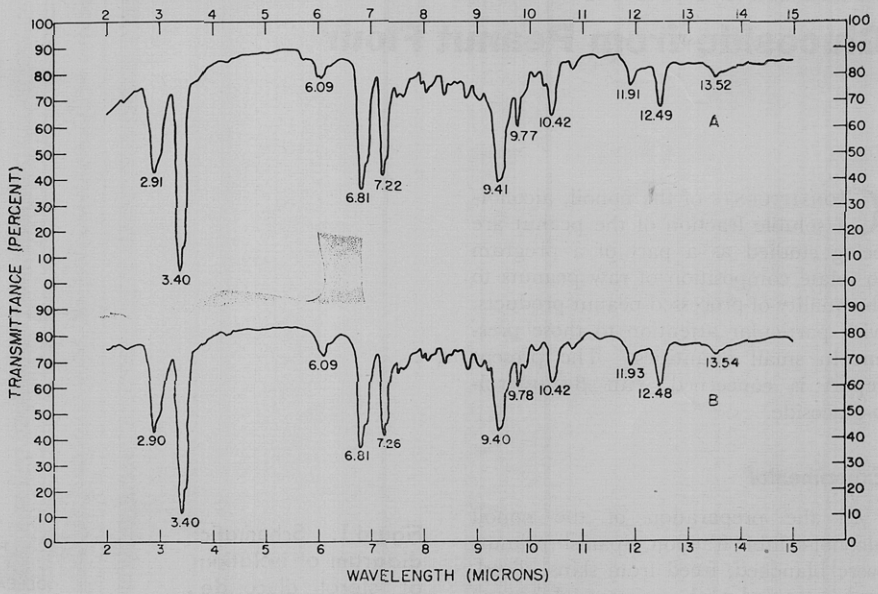

Figure 4. Infrared absorption spectra

A. Aglycone from glucoside isolated from peanut flour
B. Authentic β-sitosterol

It was then taken up in boiling methanol and crystallized from this solvent.

The crystalline product melted between 285° and 289° C. with decomposition. It gave a positive Molisch test, a red color with concentrated sulfuric acid, a positive Lieberman-Burchard reaction, and a negative ninhydrin reaction. It was readily soluble in pyridine and amyl alcohol, and slightly soluble in acetone, ethyl alcohol, and methanol. The melting point was not depressed on admixture with an authentic sample of β-sitosterol-D-glucoside. Analysis: C, 72.5; H, 10.5. Calculated for β-sitosterol-D-glucoside: C, 72.8, H, 10.5.

The glucoside was also isolated by

column chromatography. A portion of solution E was lyophilized, and 100 mg. of the dried substance was placed on a silicic acid column (1 × 9 cm.). The column was developed with benzene–ethyl ether–ethyl alcohol, 50:15:2. The eluted fractions were collected and weighed. Progress in the fractionation was followed by means of glass fiber paper chromatograms, using the equipment and procedures described by Dieckert and Reiser (*4*) and modified by Dieckert and Morris (*2*). The glass fiber paper (Reeve Angel, No. x-934-AH) was impregnated with silicic acid (*1*). The developing solvent for the paper chromatograms was the same as that used to develop the silicic column. After the solvent had evaporated from the paper, the chromatogram was sprayed with concentrated sulfuric acid and heated, first mildly, over a hot plate for the development of color, and finally more vigorously to char the organic substances to produce black spots on the white background (*5*).

About the first 8% of the dried eluate from the column contained substances that moved with the solvent front on the glass paper. The next 15% showed a spot well separated from both the front and the origin. This spot showed a pink to violet color when the chromatogram was sprayed with concentrated sulfuric acid and heated mildly. The R_f for the spot corresponded to that produced by an authentic specimen of β-sitosterol-D-glucoside. In Figure 2, *A* is a chromatogram of solution E, *B* one of authentic β-sitosterol-D-glucoside, *C* one of the glucoside separated from the silicic acid column, and *D* one of the glucoside crystallized from solution E.

The glucoside was hydrolyzed according to the method of Power and Salway (*6*). Five milligrams was added to a mixture composed of 3 ml. of amyl alcohol, 5 ml. of 15% aqueous HCl, and 25 ml. of methanol. The resulting solution was refluxed for 6 hours and the amyl alcohol removed by steam distillation.

The aglucone was removed from the aqueous solution by extraction with diethyl ether. It was then crystallized from methanol (m.p. 136–38° C.), with no depression of the melting point on admixture with authentic β-sitosterol. Analysis: C, 83.0; H, 12.3. Calculated for β-sitosterol: C, 83.99; H, 12.15.

The aqueous solution was treated with an excess of silver carbonate and filtered. The filtrate was chromatographed on glass fiber paper which had been impregnated with monopotassium phosphate in accordance with the method of Dieckert and Morris (*3*). The R_f value for the sugar obtained from the hydrolyzate was the same (0.78) as that obtained with D-glucose when the chromatogram was developed with acetone–*n*-propyl alcohol–water, 50:40:10.

The infrared spectra (potassium bromide disk method) of the glucoside isolated from peanut flour and of authentic β-sitosterol-D-glucoside showed the two compounds to be identical (Figure 3). The infrared spectra of the aglycone of the glucoside from peanut flour and of authentic β-sitosterol showed these compounds to be identical also (Figure 4).

Acknowledgment

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CAROTENOID EPOXIDE DETECTION

An Improved Test for Carotenoid Epoxides

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WHEN ETHER solutions of certain carotenoids are shaken with concentrated hydrochloric acid, the acid layer turns blue (*7*). Most of these carotenoids are epoxides (a number of which occur in nature) or their furanoid transformation products, which are referred to here as 5,6-epoxides and 5,8-epoxides, respectively, following the nomenclature of Goodwin (*5*). In general, a much deeper blue color forms with diepoxides than with monoepoxides (*7*); hydroxyl groups increase the stability and intensity of the blue color (*6*). An improved test has been developed for detection of carotenoid epoxides and for differentiation of diepoxides and monoepoxides; a modification of this test can be used to distinguish 5,6-epoxides from 5,8-epoxides.

Experimental

Materials. The carotenoid fractions used were obtained from known sources, especially oranges (*4*), cling peaches (*1*), and leaves (*3*); capsanthin and capsorubin were obtained from red garden peppers, and cryptoxanthin mono- and diepoxides from Meyer lemons (*2*). The identifications were based on spectral absorption curves, and on behavior in countercurrent distribution, chromatography, and acid treatment. A sample of β-apo-2-carotenal was kindly provided by the late G. F. Siemers, Hoffman-La Roche Inc., Nutley, N. J.

Observations on the Hydrochloric Acid–Ether Test. Hydrochloric acid–ether tests were made by the procedure previously described (*4*) on many carotenoid fractions obtained from various fruits and leaves. A deep blue color was formed by violaxanthin (zeaxanthin 5,6,5′,6′-diepoxide), its 5,8-epoxide isomerization products, and some polyol monoepoxides. In other cases (such as cryptoxanthin diepoxides and neoxanthin) a definite, but weaker color was formed, and in still other cases (mutatoxanthins and cryptoxanthin monoepoxides) the blue color was pale or uncertain. Capsanthin (a diol ketone) gave a light salmon color in the hydrochloric acid layer, capsorubin (a diol