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The Sterol and Carbohydrate Constituents of the Walnut (*Juglans regia*)[1]

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The sterol and carbohydrate constituents of the walnut have been isolated and identified as β-sitosterol, β-sitosteryl-D-glucoside, and sucrose.

INTRODUCTION

The sterol and carbohydrate constituents of the English walnut (*Juglans regia*) have been investigated in this Laboratory as part of a comprehensive study of the components of the walnut pellicle (skin). The only recorded investigation of these walnut constituents is that of Menozzi and Moreschi[2] who isolated an unidentified phytosterol from walnut oil. The phytosterol was characterized by the preparation of several derivatives and assigned the formula $C_{27}H_{45}OH \cdot H_2O$.

In this investigation two sterols were isolated from the ether-soluble fraction of methanol extracts of the walnut pellicle. Analysis of the acetate and *p*-nitrobenzoate of the lower-melting sterol, m.p. 139°, established its formula as $C_{29}H_{49}OH$. It was identified as β-sitosterol by mixture melting point comparison with authentic β-sitosterol and by the close agreement of the melting points of its derivatives with the literature values for the corresponding β-sitosterol derivatives. The second sterol was almost insoluble in the usual organic solvents and gave a positive Molisch carbohydrate test. Analysis of the compound and its *p*-nitrobenzoate established its molecular formula as $C_{35}H_{56}O_2(OH)_4$. Furthermore the compound was readily hydrolyzed by acids to give β-sitosterol. From these data it is apparent that the compound is a β-sitosteryl glycoside. Its melting point (m.p. 296°) and that of its acetate (m.p. 170°) agree closely with the values recorded by Swift[3] for β-sitosteryl-D-glucoside (m.p. 298°) and its tetraacetate (m.p. 171°). Direct comparison by mixture melting point of the walnut glycoside and authentic β-sitosteryl-D-glucoside proved their identity.

Paper chromatograms of aqueous and aqueous methanol extracts of walnut kernels and pellicles indicated the presence of only one free carbohydrate in walnuts. It crystallized from concentrated methanol extracts and was identified as sucrose by mixture melting point, optical rotation, paper chromatography, and by its conversion into glucosazone.

EXPERIMENTAL

Isolation of β-sitosterol and β-sitosteryl-D-glucoside. Finely powdered walnut pellicles (1500 g.) were extracted with low-boiling petroleum ether for 24 hours and then with methanol for 48 hours. The methanol extract was concentrated and added slowly to ether (3000 cc.). After standing overnight the clear ether solution was decanted from the precipitated phenols,[4] washed with two 300-cc. portions of water, dried over sodium sulphate, and evaporated to an oil. The oil was dissolved in 500 cc. of warm methanol. Saturated methanolic lead acetate (300 cc.) was added to the methanol solution and the precipitated lead salts were filtered. The filtrate was treated with hydrogen sulphide to remove excess lead, and the lead sulphide was filtered. The filtrate was concentrated to about 500 cc. and allowed to stand. The sterols separated in clusters of colorless needles. The crystalline mixture was collected, warmed with *n*-hexane, and filtered. The hexane-insoluble residue consisted of β-sitosteryl-D-glucoside (0.08 g.). Evaporation of the hexane filtrate gave colorless crystals of β-sitosterol (0.15 g.).

β-Sitosterol. The walnut sterol identified as β-sitosterol crystallized from methanol in glistening plates, m.p. 137–139° (lit.[3] for β-sitosterol, 137–138°), which gave a positive Lieberman-Burchard test. The melting point was not depressed on admixture with authentic β-sitosterol.[5]

β-Sitosteryl acetate. The sterol (50 mg.) was acetylated in the usual way with acetic anhydride and sodium acetate. The acetate crystallized from methanol in colorless needles, m.p. 121–121.5° (lit.[6] for β-sitosteryl acetate, 120–121°).

Anal. Calc'd for $C_{31}H_{52}O_2$: C, 81.5; H, 11.5. Found: C, 81.4; H, 11.4.

β-Sitosteryl p-nitrobenzoate. A solution of the sterol (35 mg.) and *p*-nitrobenzoyl chloride (0.1 g.) in pyridine (0.2 cc.) was heated on a steam-bath for 5 minutes. The solution was cooled, diluted with water, and extracted with ether. The ether extract was washed successively with dilute sodium bicarbonate, hydrochloric acid, and water, dried and evaporated. The residue was crystallized from methanol-acetone. β-Sitosteryl *p*-nitrobenzoate separated in colorless felted needles, m.p. 185–186° (lit.[6] for β-sitosteryl *p*-nitrobenzoate, 187°).

Anal. Calc'd for $C_{36}H_{53}NO_4$: C, 76.7; H, 9.5; N, 2.5. Found: C, 76.6; H, 9.4; N, 2.5.

β-Sitosteryl-D-glucoside. The crude hexane-insoluble sterol glycoside was recrystallized from large volumes of acetone-methanol. It separated in colorless needles, m.p. 296°, undepressed on admixture with authentic β-sitosteryl-D-glucoside. The glycoside gave a positive Lieberman-Burchard test and a positive Molisch test.

Anal. Calc'd for $C_{35}H_{60}O_6$: C, 72.8; H, 10.5. Found: C, 72.3; H, 10.5.

(1) Financial support for this work was provided by the California Walnut Growers Association.

(2) A. Menozzi and A. Moreschi, *Atti accad. Lincei*, **19, I,** 126–129 (1910); *Chem. Abstr.*, **4,** 2455 (1910).

(3) L. J. Swift, *J. Am. Chem. Soc.*, **74,** 1099 (1952).

(4) L. Jurd, *J. Am. Chem. Soc.*, **78,** 3445 (1956).

(5) In certain cases different sitosterols fail to show a depression in the mixed melting point. However, enough derivatives of the walnut sterol were prepared to make its identity as β-sitosterol certain.

(6) F. E. King and L. Jurd, *J. Chem. Soc.*, 1192 (1953).

β-Sitosteryl-D-glucoside tetra-p-nitrobenzoate. A mixture of the glucoside (30 mg.), *p*-nitrobenzoyl chloride (0.15 g.), and pyridine (0.3 cc.) was heated on the steam-bath for 10 minutes. After dilution with water the solid *p*-nitrobenzoate was collected, washed with dilute sodium bicarbonate and hydrochloric acid, and recrystallized from acetone-methanol. β-Sitosteryl-D-glucoside tetra-*p*-nitrobenzoate separated in colorless needles, m.p. 275–277°.

Anal. Calc'd for $C_{63}H_{72}N_4O_{18}$: C, 64.4; H, 6.2; N, 4.8. Found: C, 64.4; H, 6.20; N, 4.7.

β-Sitosteryl-D-glucoside tetraacetate. The glucoside was acetylated with acetic anhydride and sodium acetate. The acetate crystallized from methanol in colorless glistening needles, m.p. 170°.

Hydrolysis of β-sitosteryl-D-glucoside. The glucoside (20 mg.) was heated under reflux for 2 hours with ethanol (10 cc.) containing concentrated sulphuric acid (0.2 cc.). The glucoside gradually dissolved. The solution was concentrated to about 4 cc., diluted with water, and extracted with ether. The ether solution was washed with water, dried, and evaporated to a crystalline residue. Recrystallization of the residue from methanol gave β-sitosterol in colorless flakes, m.p. 137–138°, undepressed on admixture with the walnut β-sitosterol.

Isolation of sucrose. The gum obtained by the addition of the concentrated methanol extract to ether was extracted with hot acetone (4 × 800 cc.) to remove phenolic material. The acetone-insoluble residue was dissolved in boiling methanol (500 ml.), and the solution was filtered and allowed to stand for several days. Large colorless crystals of sucrose (4.7 g.) thereby separated. Larger quantities of sucrose were obtained from walnut kernels. The sliced kernels (100 g.) were extracted in a Soxhlet with ether for eight hours and then with methanol for 40 hours. The methanol extract, concentrated to 25 cc. and allowed to stand 24 hours, deposited crystalline sucrose (1.9 g.).

Identification of sucrose. The walnut carbohydrate crystallized from methanol in colorless prisms, m.p. 179–184°, undepressed on admixture with authentic sucrose. In water, $[\alpha]_D^{20}$ +65.7° (lit. for sucrose, in water $[\alpha]_D^{20}$ +66.37°). It did not reduce Fehling's solution until hydrolyzed with acid. The carbohydrate heated with phenylhydrazine and sodium acetate in dilute acetic acid gave a yellow osazone which was recrystallized from methanol. It separated in yellow needles, m.p. 203°, undepressed on admixture with glucosazone prepared similarly from sucrose. The walnut sugar and sucrose had identical R_F values in *tert*-butanol/water/formic acid (69.5:29.5:1, v/v) (R_F 0.35); phenol/water/ammonia (77.5:21.5:1, w/v/v) (R_F 0.30); and *n*-butanol/acetic acid/water (20:6:15, v/v) (R_F 0.27).

Paper chromatograms of aqueous and methanolic extracts of walnut pellicle and kernel did not indicate the presence of any carbohydrate other than sucrose. More sensitive methods might reveal trace amounts of other carbohydrates.

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