Isolation of β-Sitosterol, β-Sitosteryl-D-Glucoside, and Palmitic Acid from Coastal Bermuda Grass and Orchard Grass

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β-Sitosterol and β-sitosteryl-D-glucoside were isolated from coastal Bermuda grass and from orchard grass, and identified by infrared spectra and physical constants on the sterol and the acetate. Palmitic acid was isolated from the saponifiable fractions. No saponin was detected.

BERMUDA grass and orchard grass were investigated for saponins by means of the procedure used to isolate saponin from ladino clover (1). Material was obtained which gave a Liebermann-Burchard color test suggesting sterols rather than saponin. A study of this material showed that it contained β-sitosterol, β-sitosteryl-D-glucoside, and palmitic acid. Repeated experiments were carried out on 1-Kg. quantities of dehydrated meal to accumulate enough material for identification. Results were practically identical with either of these grasses. No saponin was obtained by this method from these grasses.

EXPERIMENTAL

The dehydrated meal (1 Kg.) was soaked with about 4 L. of warm water for 2 hours. The water was filtered through cloth with suction and discarded. The hydrated meal was covered with 95% alcohol (5–6 L.) and allowed to stand for a day or more. The extract was filtered with suction, and the alcohol extraction was repeated. The combined alcohol extracts were clarified with charcoal (10 Gm./L.), and concentrated to about 1.5 L. The concentrate was extracted with ether in a separator. The ether was evaporated and the residue was saponified with alcoholic potassium hydroxide. The alkaline mixture was extracted with ether, and the ether solution was evaporated to near dryness.

β-Sitosteryl-D-glucoside.—To the unsaponifiable residue from the ether extract, about 100 ml. of skellysolve-B was added. A small quantity of the material was insoluble and was filtered. The residue was washed with acetone, leaving a white product, m.p. 280°. Yield, 90 mg./Kg. dry meal. The material recrystallized from methanol, m.p. 296° (with dec.), showed an infrared spectrum that was in good agreement with that presented by Morris and Lee (2) for β-sitosteryl-D-glucoside.

Hydrolysis of the glucoside with 2% sulfuric acid by the method of Thornton, *et al.* (3), gave β-sitosterol, m.p. 137°, with an infrared spectrum in good agreement with that reported in the literature (2), and glucose which was detected by paper chromatography after the acid had been removed by barium carbonate.

β-Sitosterol.—The skellysolve-B-soluble part of the unsaponifiable material was put on a column of deactivated alumina. The sterol was eluted with 3% acetone in skellysolve-B, and the solvent removed. The sterol crystallized from methanol yielded about 200 mg./Kg. of dry meal, m.p. 137° [lit. 136–137° (4)]. The infrared spectrum was in agreement with that reported (2) for β-sitosterol.

β-Sitosteryl Acetate.—The sterol was heated with acetic anhydride for 30 minutes. The mixture was poured into ice water, and the fluffy product was filtered and washed with water. The dried product was recrystallized from methanol, m.p. 127°; $[\alpha]_D^{25}$ − 38.4° (in chloroform, c = 1.11, *l* = 2), [lit. (4) $[\alpha]_D$ − 38° in chloroform].

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.52, H, 11.48. Found: C, 81.6, H, 11.4.

Palmitic Acid.—After ether extraction of the sterols, the aqueous saponifiable portion was acidified with hydrochloric acid and again extracted with ether. The acid was washed out of the ether extract with water and the ether solution was clarified with charcoal. The solution was concentrated to near dryness, then dissolved in methanol. On standing, a white crystalline solid separated. Recrystallization from methanol gave white flakes, m.p. 63°. Titration with 0.1*N* sodium hydroxide gave an equivalent weight of 260 (palmitic acid, 256.32). A gas chromatogram showed that the retention times of this material and that of authentic palmitic acid were identical. Likewise, the infrared spectra of this material and that of authentic palmitic acid were identical.

A minute quantity of material, m.p. about 90°, was obtained before saponification of the ether extract, which suggests that at least part of the sterol occurred as β-sitosteryl palmitate.

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