

Isolation of α -Spinasteryl-D-glucoside and α -Spinasterol from Alfalfa

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α -Spinasteryl-D-glucoside was isolated from alfalfa and identified by infrared spectra and by identification of its products of hydrolysis. α -Spinasterol was isolated from the unsaponifiable fraction and palmitic acid from the saponifiable.

ALTHOUGH α -spinasterol was previously isolated from alfalfa by Fernholz and Moore (1) the glucoside has never been mentioned before. Blair *et al.* (2) in a study of sterols of alfalfa, stated that combined sterols were not detected at any time during its growth up to early bloom. In the process of isolation of the water-insoluble or soya-type saponin from alfalfa, an ether-soluble sterol fraction was obtained as a by-product. The same treatment that yielded β -sitosterol and β -sitosteryl-D-glucoside from coastal Bermuda grass and orchard grass (3) yielded α -spinasteryl-D-glucoside and α -spinasterol from alfalfa.

EXPERIMENTAL

One-kilogram samples of alfalfa meal (or pellets) were soaked with 10 L. of warm water for 2 hours. An extract was filtered through cloth with suction for the isolation of water-soluble saponin. The remaining hydrated meal was allowed to stand for 1 day or more in 95% alcohol (7 L.). The alcohol extract was filtered with suction, clarified with charcoal (10 Gm./L.), and concentrated to about 1.5 L. The concentrate was extracted with ether, the ether evaporated, and the residue saponified with alcoholic potassium hydroxide. Water was added and the mixture was extracted with ether; the alkali was washed out with water, and the ether extract was evaporated to near dryness.

α -Spinasteryl-D-glucoside.—About 100 ml. of skellysolve-B was added to the unsaponifiable residue. The insoluble portion was collected on a filter and washed with acetone, leaving a white powder, m.p. 290°. Yield varied from 30–55 mg./Kg. of dehydrated meal. The material recrystallized from pyridine-methanol, m.p. 290–292° (with dec.), $[\alpha]_D^{25} - 32.2^\circ$ (in pyridine, $c = 1.754$, $l = 0.5$), showed an infrared spectrum in agreement with that presented by Obata *et al.* (4) for this glucoside isolated from sugar beet pulp.

Anal.—Calcd. for $C_{35}H_{58}O_6$; C, 73.12; H, 10.17. Found: C, 72.8; H, 10.3.

Hydrolysis of the glucoside with 2% sulfuric acid by the method of Thornton *et al.* (5) gave α -spinasterol, m.p. 167°, with an infrared spectrum in

agreement with that reported by Obata *et al.* (4) and glucose which was detected by paper chromatographic comparison with known glucose.

α -Spinasteryl-D-glucoside Tetraacetate.—The glucoside (25 mg.) was dissolved in 5 ml. of pyridine and 5 ml. of acetic anhydride was added. The mixture was refluxed for 1 hour, then poured into ice water. The solid material was filtered out, washed with water, and recrystallized from alcohol, m.p. 168°, [lit. 168° (4)].

Anal.—Calcd. for $C_{43}H_{70}O_{10}$: C, 69.51; H, 8.95. Found: C, 69.1; H, 8.92.

α -Spinasterol.—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 was evaporated and recrystallized from methanol, m.p. 167°. The sterol gave a positive Lieberman-Burchard reaction. The infrared spectrum was in agreement with that of α -spinasterol (4). The yield varied from 20–70 mg./Kg. of dehydrated meal.

α -Spinasteryl Acetate.—The sterol (120 mg.) was heated on the steam bath with 10 ml. acetic anhydride for about 1 hour. The mixture was poured into ice water and the fluffy product was filtered, washed with water, dried, and recrystallized from alcohol; yield 130 mg., m.p. 181–183°, [lit. 183° (1)].

Anal.—Calcd. for $C_{31}H_{50}O_2$: C, 81.87; H, 11.09. Found: C, 81.64; H, 11.1.

α -Spinasteryl Benzoate.—The sterol (250 mg.) was dissolved in pyridine, and 0.5 ml. benzoyl chloride was added. The mixture was heated on the steam bath for 2 hours, cooled, and poured into cold dilute sodium bicarbonate solution. It was filtered after standing overnight in the cold. The residue crystallized from chloroform and absolute alcohol, m.p. 199–200°; $[\alpha]_D^{25} + 3.8^\circ$ (in chloroform, $c = 3.68$, $l = 0.5$).

Anal.—Calcd. for $C_{36}H_{52}O_2$: C, 83.65; H, 10.15. Found: C, 83.6; H, 10.1.

Palmitic Acid.—The aqueous saponifiable fraction from the sterol separation was acidified with hydrochloric acid and extracted with ether. The acid was washed out with water, and the ether solution was clarified with charcoal. The solution was concentrated to near dryness, then dissolved in methanol. Recrystallization from methanol gave white flakes, m.p. 63°. Titration with 0.1 N sodium hydroxide gave an equivalent weight of 258 (palmitic acid, 256.32). The infrared spectra of this material and that of authentic palmitic acid were identical.

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