[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CONNECTICUT]

STEROIDS OF THE VIRGINIA SNAKEROOT, *Aristolochia serpentaria*[1]

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The first isolation of a steroid from a species of Aristolochia was reported by Hesse in 1892 (1). Physical data for the palmitate and acetate obtained from *Aristolochia argentina* were reported in a later paper (2) and led Hesse to assign the formula $C_{26}H_{44}O$ for the free sterol. An examination of the data however reveals a closer agreement with the formula $C_{29}H_{50}O$, the latter being more likely in light of modern knowledge of plant sterol structures. Krishnaswamy *et al.* (3) have reported the presence of a "phytosterol", (m.p. 137–138°) and a steryl glycoside (m.p. 285–290°) in extracts obtained from *Aristolochia indica.*

TABLE I

COMPARISON OF STEROLS AND STEROL DERIVATIVES

COMPOUND	β-SITOSTEROL[a]		*A. Serpentaria* STEROL	
	M.P., °C.	$[\alpha]_D$	M.P., °C.	$[\alpha]_D$
Sterol	139–140	−37.0	139–140	−38.0
Steryl acetate	127–128	−41.0	130–132	−42.5
Steryl benzoate	146–147	−13.8	144–145	−13.9
Steryl *m*-dinitrobenzoate	202–203	−10.4	209–210	−12.7
Stanol	144–145	+25.0	142–143	+25.4
Stanyl acetate	137–138	+14.0	136–138	+12.8

[a] Data from *Elsevier's Encyclopedia of Organic Chemistry*, Elsevier Publishing Co., Inc., New York, N. Y., Vol. **14** (1940).

It became possible to carry out a more complete characterization of the steroids of the genus *Aristolochia* when extracts of the root of *A. serpentaria* were made available to the authors through the generosity of Professor Earl C. Spaeth. A petroleum ether extract of 100 lbs. of roots yielded 1997 g. of viscous residue. Saponification gave 495 g. of unsaponifiable matter from which a sterol fraction (22.25 g.) was isolated by precipitation with digitonin. After several crystallizations, the sterol melted at 139–140°. Preparation of several derivatives and hydrogenation to the stanol characterized the sterol as β-sitosterol. (Table I).

An ethyl ether extract (324 g.) obtained by subsequent treatment of the root material, was saponified, and 2.7 g. of a high-melting steryl glycoside was isolated during ether extraction of the saponification mixture. The tetraacetate gave analytical values in agreement with a C_{29} sterol and a six carbon sugar. Hydrolysis of the glycoside permitted the identification of the sterol as β-sitosterol, which was characterized by comparison with the sterol previously isolated from

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[2] Taken from a thesis submitted by Vincent D. Celentano in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

the petroleum ether extract. Although the amount of material available did not permit the isolation and unequivocal identification of the hexose moiety, the *Aristolochia* glycoside was shown to be a β-glucoside by comparison with the compound prepared by the reaction of acetobromoglucose and β-sitosterol (Table II). The nature of the glucosidic linkage was predicted on the basis of molecular rotation differences. Data obtained by Linstead (4) for the α- and β-tetraacetyl-glucosides of cholestanol show an increment of −57 for a β-linked tetraacetyl-glucosyl group and an increment of +727 for the α-form. The value of −27 calculated for the *Aristolochia* derivative suggested a β-glucosidic link. (Table III). The glycoside of *Aristolochia serpentaria* is identified therefore as β-(β-

TABLE II

COMPARISON OF STERYL GLUCOSIDES

COMPOUND	GLUCOSIDE M.P., °C.	TETRAACETATE		
		M.P., °C.	$[\alpha]_D$	$[\alpha]_D(C_5H_5N)$
A. serpentaria	295–297	167.5–168.5	−24.2	−27.4
β-(β-Sitosteryl)-D-glucoside	298–299	167.5–168.5	−23.7	−26.5
Citrus sinensis	298	171		−33.7

TABLE III

COMPARISON OF MOLECULAR ROTATION DIFFERENCES

COMPOUND	$[\alpha]_D$	M_D	ΔM_D
Cholestanol	+24	+93	
α-Cholestanyl-D-glucoside tetraacetate	+114	+820	+727
β-Cholestanyl-D-glucoside tetraacetate	+5	+36	−57
β-Sitosterol	−37	−153	
Aristolochia glycoside tetraacetate	−24.2	−180	−27

sitosteryl)-D-glucoside. The tetraacetate showed no depression in melting point when mixed with a sample of tetraacetate of β-sitosteryl glucoside isolated from the juice of *Citrus sinensis*.[3]

EXPERIMENTAL

Melting points were taken with Anschütz total immersion thermometers. Optical rotations were taken in a 2-dm. tube, the sample being dissolved in 4.99 ml. of chloroform. The analyses were carried out by the Laboratory of Microchemistry, Teaneck, New Jersey.

A. Petroleum ether extract. The petroleum ether extract of 100 lbs. of dried crushed roots was distilled under reduced pressure at 40° giving 1997 g. of a dark red viscous oil. The oil was steam-distilled and then dried by co-distillation with benzene giving 1886 g. of dry residue. The residue was saponified, in 400-g. portions, by refluxing for two hours with 20% potassium hydroxide in 75% ethanol. The ethanol was removed under reduced pres-

[3] The authors wish to express their gratitude to Dr. L. J. Swift of the Citrus Products Station, USDA, Winter Haven, Florida, for samples of the glucoside and the tetraacetate.

sure and the mixture was diluted with two volumes of water. The aqueous solutions were exhaustively extracted with ethyl ether and the ether extracts dried over sodium sulfate. The ether extracts were combined and the solvent was removed by distillation yielding 495 g. of red-brown nonsaponifiable matter.

Isolation of the sterol. The nonsaponifiable matter was taken up in hot ethanol, cooled, filtered free of a small amount of non-steroid precipitate, and heated to boiling. To the boiling solution was added 1% digitonin (in ethanol), and the mixture was allowed to stand for 48 hours. The insoluble digitonide was filtered off and the digitonin treatment was repeated until no further precipitation occurred. In this manner 97.3 g. of precipitate was collected. The crude tan digitonide was digested with anhydrous ethyl ether to remove the coloring matter and then split by the method of Bergmann (5) to give 22.25 g. of white crystalline sterol. The sterol was recrystallized from chloroform-methanol to a constant melting point, 139–140°; $[\alpha]_D^{24}$ −38° (65.5 mg.; α, −0.99°).

Anal. Calc'd for $C_{29}H_{50}O \cdot CH_3OH$: C, 80.65; H, 12.18.
Found: C, 80.71; H, 11.80.

Steryl acetate. One gram of sterol was acetylated by refluxing with 15 ml. of acetic anhydride. The acetate was recrystallized from absolute methanol; m.p. 130–132°; $[\alpha]_D^{23}$ −42.5° (55.5 mg.; α, −0.946°). Perbenzoic acid titration gave 1.02 double bonds.

Anal. Calc'd for $C_{31}H_{52}O_2$: C, 81.51; H, 11.48.
Found: C, 80.98; H, 11.14.

Steryl benzoate. The sterol was treated with benzoyl chloride in pyridine. The benzoate was precipitated with methanol and recrystallized from ethyl acetate; m.p. 144–145°; $[\alpha]_D^{27}$ −13.9° (71.2 mg.; α, −0.39°).

Anal. Calc'd for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49.
Found: C, 82.74; H, 10.27.

Steryl m-dinitrobenzoate. The derivative was prepared as described for the benzoate, and recrystallized from chloroform-methanol, m.p. 209–210°; $[\alpha]_D^{26}$ −11.75° (54.0 mg.; α,−0.25°).

Anal. Calc'd for $C_{36}H_{52}N_2O_6$: C, 71.01; H, 8.61.
Found: C, 70.96; H, 8.83.

Stigmastanyl (β-sitostanyl) acetate. The steryl acetate was hydrogenated in glacial acetic acid at room temperature with platinum oxide catalyst. A Liebermann-Burchard test was negative. The product was recrystallized from chloroform-methanol, m.p. 136–138°; $[\alpha]_D^{18}$ +12.84° (52.0 mg.; α, +0.26°).

Anal. Calc'd for $C_{31}H_{54}O_2$: C, 79.43; H, 11.61.
Found: C, 79.78; H, 11.40.

The stanyl acetate was saponified in the usual manner to give stigmastanol, (β-sitostanol), m.p. 142–143°; $[\alpha]_D^{25}$ +25.38° (74.5 mg.; α, +0.76°).

B. Ethyl ether extract. The dried extract (324 g.) was saponified by refluxing with 10 volumes of 15% potassium hydroxide in 75% ethanol. The mixture was diluted with twice its volume of water and extracted with 16 liters of ether. The ether was washed with water to remove excess alkali and, during the washing procedure, the solid interfacial precipitate which appeared was removed. The ether solution was concentrated to a volume of 1200 ml. and filtered free of more solid. The combined solids were washed with chloroform and air-dried. A total of 2.7 g. was obtained. The material decomposed at 263° and was only slightly soluble in chloroform and boiling ethanol.

Steryl glycoside tetraacetate. The interface material was dissolved in 20 ml. of dry pyridine and refluxed for one hour with 13 ml. of acetic anhydride. The solvent was removed *in vacuo* and the residue was taken up in boiling ethanol, filtered while hot, and cooled for 24 hours at 5°. The white crystalline precipitate was filtered and recrystallized several times from chloroform-methanol, m.p. 167.5–168.5°; $[\alpha]_D^{25}$ −24.2° (63.8 mg.; α, −0.62°).

When mixed with β-sitosteryl-D-glucoside tetraacetate (m.p. 170–171°), (6) the melting point was 168–169.5°).

Anal. Calc'd for $C_{43}H_{67}O_{10}$: C, 69.42; H, 9.08.
Found: C, 69.12; H, 9.15.

Saponification of the tetraacetate with alcoholic NaOH yielded the free glycoside which, after repeated crystallizations from pyridine-methanol, decomposed at 295–297° (bath preheated to 288°).

β-Sitosterol. The steryl glycoside (1.25 g.) was refluxed for ten hours with 100 ml. of ethanol containing 2 ml. of conc'd HCl. The ethanol was removed and the free sterol was recrystallized from ether, ethanol, and methanol, m.p. 139–140°; $[\alpha]_D^{25}$ −37° (77.6 mg.; α, −1.15°). A mixture melting point with the sterol isolated from the petroleum ether extract showed no depression.

The sterol was converted to the *benzoate* with benzoyl chloride in pyridine, m.p. 145–146°; $[\alpha]_D^{25}$ −12.15° (76.4 mg.; α, −0.37°).

Anal. Calc'd for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49.

Found: C, 83.15; H, 10.24.

There was no depression in melting point when the benzoate was mixed with the benzoate of the sterol isolated from the petroleum ether extract.

Synthesis of β-sitosteryl-β-D-glucoside tetraacetate. β-Sitosterol (4 g.) from the petroleum ether extract was dissolved in 100 ml. of absolute ether. Drierite (8 g.) and freshly prepared silver oxide (3 g.) were added and the mixture shaken for 30 minutes. Acetobromoglucose (4.24 g.) was added and the mixture was shaken at room temperature for 15 hours. The mixture was filtered and the ether was removed under reduced pressure. The crude tetraacetate was taken up in ether and precipitated by the addition of methanol.

The crude tetraacetate (400 mg.) was dissolved in ethanol and a solution of 10% KOH in ethanol was added. On standing at room temperature a white crystalline precipitate of steryl glycoside was formed. The mixture was heated to boiling, filtered, and washed with hot ethanol. The glycoside was purified by repeated digestion with boiling ethanol and finally dissolved in boiling pyridine and precipitated with methanol, m.p. 298–299° (bath preheated to 288°).

A solution of 70 mg. of glycoside in 3 ml. of dry pyridine was refluxed for one hour with 1 ml. of acetic anhydride. The solvent was removed by distillation under reduced pressure and the residue was dissolved in boiling methanol and filtered while hot. Recrystallization from ether-methanol and chloroform-methanol gave a product melting at 167.5–168.5°; $[\alpha]_D^{24}$ −23.7° (63.4 mg.; α, −0.61°); $[\alpha]_D^{24}$ (pyridine) − 26.4° (30.5 mg.; α, −0.32°).

Mixture melting points with the tetraacetate prepared from the natural glycoside and with the tetraacetate prepared by Swift (6) showed no depression.

Anal. Calc'd for $C_{43}H_{67}O_{10}$: C, 69.42; H, 9.08.

Found: C, 69.39; H, 9.00.

SUMMARY

Two steroids have been identified in the unsaponifiable matter of the Virginia snakeroot, *Aristolochia serpentaria.* The major component of the sterol fraction of a petroleum ether extract of the dried root material is β-sitosterol. The sterol was characterized by the preparation of the acetate, benzoate, *m*-dinitrobenzoate, and by hydrogenation to β-sitostanol (stigmastanol).

An ethyl ether extract yielded a steryl glycoside, identified as β-(β-sitosteryl)-D-glucoside. Hydrolysis yielded β-sitosterol which was characterized by comparison with the sterol isolated from the petroleum ether extract. The tetraacetate of the natural glycoside is identical with the compound prepared by reaction of the β-sitosterol with acetobromoglucose. The synthesis established the nature of the glucosidic linkage which had been predicted on the basis of differences in molecular rotation.

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