146. *The Constituents of Senega Root. Part I. α-Spinasterol.*

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The literature being notably devoid of information regarding the constituents of senega root other than senegin, an investigation of this problem has been commenced, attention being first directed to the lipoidal fraction of an alcoholic concentrate of the root. This concentrate, after removal of senegin, consisted principally of ether-insoluble material which, although sparingly soluble in water, dissolved in aqueous sodium hydroxide. Extraction of the alkaline solution with ether yielded a comparatively odourless, fatty oil moderately rich in non-saponifiable matter, which consisted of a partly crystalline, viscous mass having a highly characteristic and not unpleasant odour. Benzoylation of this material furnished a mixture, from which a product of constant m. p. 200° was isolated. By hydrolysis of this benzoate a sterol, m. p. 169—170°, was obtained, which was converted into its acetate, m. p. 184—185°, and *p*-nitrobenzoate, m. p. 213·5—215°. These three compounds are identical with α-spinasterol, its acetate, and *p*-nitrobenzoate, for samples of which the author is greatly undebted to Dr. Heyl.

α-Spinasterol, already examined by Hart and Heyl (*J. Biol. Chem.*, 1932, **95**, 311) and Larsen and Heyl (*J. Amer. Chem. Soc.*, 1934, **56**, 2663), has now been further characterised. It was recovered unchanged in 80% yield after treatment with maleic anhydride under the conditions employed for the combination of the latter with ergosterol (Windaus and Luttringhaus, *Ber.*, 1931, **64**, 850), and therefore conjugation of its two ethenoid linkages is excluded, despite the apparent nuclear similarity of the two sterols. This conclusion is supported by the ultra-violet absorption spectrum of α-spinasterol, which, though resembling that of ergosterol qualitatively, has an extinction coefficient which is only 0·5% of that of the latter substance. The possibility that this slight absorption is due to the presence in senega root of a trace of ergosterol (or other similarly constituted sterol) is being investigated. The sterol gives a transient but definite faint pink coloration with antimony trichloride in chloroform; this behaviour, however, is also observed with the sterol recovered from the maleic anhydride treatment.

In the hope of obtaining information as to the position of one or both of the unsaturated

centres, a preliminary study was made of the behaviour towards chromic anhydride and perbenzoic acid of α-spinasterol and its acetate. The free sterol was oxidised smoothly by the former reagent at room temperature to the corresponding ketone, *α-spinastadienone*, m. p. 176° (*oxime*, m. p. 253°). Similar oxidation of the acetate on the other hand gave a mixture of neutral products, from which two crystalline *substances* were isolated. The less soluble substance, $C_{30}H_{46}O_3$, m. p. 211—213°, on hydrolysis readily yielded the corresponding *alcohol*, $C_{28}H_{44}O_2$, m. p. 152°. The second product of this oxidation had m. p. 170°; the analytical data indicated the formula $C_{30}H_{46}O_4$ (or $C_{30}H_{48}O_4$). Unlike the acetate $C_{30}H_{46}O_3$, however, this substance underwent decomposition on hydrolysis, and no homogeneous product was isolated. The structures of these products are under investigation; the course of the oxidation bears a formal resemblance to that of cholesteryl acetate ($C_{29}H_{48}O_2 \longrightarrow C_{29}H_{46}O_3 + C_{29}H_{48}O_4$; Windaus and Kirchner, *Ber.*, 1920, **53**, 614) and α-ergostenyl acetate ($C_{30}H_{50}O_2 \longrightarrow C_{30}H_{48}O_3 + C_{30}H_{48}O_4$; Heilbron, Simpson, and Wilkinson, J., 1932, 1699).

Titrations of α-spinasterol with perbenzoic acid (Larsen and Heyl, *loc. cit.*) show that the oxygen equivalent of two double bonds is ultimately taken up. It has now been found that treatment of the sterol with this reagent in approximately equimolecular proportion furnishes *α-spinasterol oxide*, m. p. 165°, in good yield. The same product is obtained by similar treatment of α-spinasteryl acetate, followed by saponification of the resultant *α-spinasteryl acetate oxide*, m. p. 159°.

In many of its properties α-spinasterol stands in marked contrast to the sterols which have hitherto been investigated in detail. Attempts, for example, to hydrate α-spinasterol oxide under a variety of conditions (compare Morrison and Simpson, J., 1932, 1710; Coffey, Heilbron, and Spring, *J.*, 1936, 738; Fernholz, *Annalen*, 1934, **508**, 215) have proved unsuccessful, whereas the oxides of cholesterol, sitosterol, stigmasterol, and α-dihydrofucosterol are all more or less readily converted into the corresponding triols. A further difference between the sterols cited and α-spinasterol is the fact that the latter forms neither a di- nor a tetra-bromide. This was observed by Larsen and Heyl (*loc. cit.*) and has been confirmed in this laboratory (see experimental). Thirdly, the melting points of the esters of α-spinasterol are considerably higher than those of the other sterols, and the characteristic high lævorotation of sterols containing a 5 : 6-double bond is not exhibited by α-spinasterol (see Table I). Lastly, the absorption spectrum of α-spinastadienone shows only two extremely faint bands at 2520 A. and 2440 A., whereas ketones derived from $\Delta^{5:6}$-unsaturated sterols, *e.g.*, cholestenone (Menschick, Page, and Bossert, *Annalen*, 1932, **495**, 225) and α-fucostenone (Coffey, Heilbron, and Spring, *loc. cit.*), exhibit the intense absorption at about 2450 A. characteristic of αβ-unsaturated ketones.

The possibility that these striking anomalies might be ascribable to the presence of a pentacyclic (triterpenoid) skeleton in the molecule of α-spinasterol is difficult to reconcile

TABLE I.

	Sterol.		Acetate.		Propionate.	Benzoate.
	M.p.	$[\alpha]_D$.	M.p.	$[\alpha]_D$.	M.p.	M.p.
Cholesterol	148°	−37·8°	114°	−43° [1]	98°	150°
Sitosterol	137	−26·7 [2]	127	−36 [3]	108 [2]	145 [2]
Stigmasterol	170	−45 [4]	141	−50·3 [5]	122 [4]	160 [4]
Fucosterol	124	−38·4 [6]	119	−43·8 [6]	105 [6]	120 [6]
α-Spinasterol	170	− 1·8 [7]	185	− 4·7 [7]	153 [7]	201 [7]

[1] Bergman, *J. Biol. Chem.*, 1934, **107**, 527. [2] Burian, *Monatsh.*, 1897, **18**, 551. [3] Anderson, Shriner, and Burr, *J. Amer. Chem. Soc.*, 1926, **48**, 2987. [4] Windaus and Hauth, *Ber.*, 1906, **39**, 4378. [5] Anderson and Shriner, *J. Amer. Chem. Soc.*, 1926, **48**, 2976. [6] Heilbron, Phipers, and Wright, J., 1934, 1572. [7] Hart and Heyl, *loc. cit.*

with the properties of the compound. Thus its isolation (together with the isomeric β-spinasterol) *via* the digitonide has been described by Heyl and Larsen (*J. Amer. Pharm. Assoc.*, 1933, **22**, 510). Furthermore the physical constants of the spinastenols and of spinastanol (Larsen and Heyl, *loc. cit.*) are typically those of hydrogenated sterols. Finally, the analytical data are in markedly better agreement with the requirements of a tetra-

cyclic, rather than a pentacyclic, nucleus. There are therefore reasonable grounds for regarding α-spinasterol as a true sterol. The anomalies noted are thus presumably due to a distribution of double bonds in α-spinasterol not hitherto encountered in sterols of known constitution, and lead to the provisional suggestion that this sterol differs from other sterols in that it does not contain the 5 : 6-double bond characteristic of the latter.

Experimental.

(Melting points uncorrected; specific rotations in chloroform.)

Isolation of α-Spinasteryl Benzoate.—Senega root (1 part) was digested thrice with boiling alcohol (5 parts), and the extract filtered hot. The crude senegin which separated on cooling was removed, and the filtrate concentrated as completely as possible under reduced pressure. The resultant soft gum was dissolved in sodium hydroxide (3 l. of 4% solution per 2 kg. of gum) by continued shaking at room temperature, and the solution after slight dilution with water was extracted with ether for 3 days in a continuous-extraction apparatus. The extract was filtered from a small amount of glycosidic material, and the residual fatty oil (1 part) was refluxed for 3 hours with potassium hydroxide in 90% alcohol (10 parts of 10%). Most of the alcohol was removed, and the residue was then largely diluted with water and extracted with ether. Evaporation of the washed and dried extract gave a semi-crystalline mass (7—8% of the fatty oil), which was dissolved in its own weight of pyridine and treated with the same amount of benzoyl chloride. After standing over-night, the product was decomposed with ice and sulphuric acid and worked up by ether-extraction, etc. Digestion of the dry residue with a little acetone gave a crystalline product, which was recrystallised once from acetone and then repeatedly from ethyl acetate, large plates of the benzoate, m. p. 199·5—200·5°, being finally obtained (2·9 g. from 630 g. of the fatty oil) (Found: C, 83·9; H. 10·1. Calc. for $C_{35}H_{50}O_2$: C, 83·6; H, 10·0%).

α-Spinasterol.—The above benzoate was refluxed for 2 hours with 5% alcoholic potassium hydroxide; the sterol, which separated from alcohol in small plates, had m. p. 169—170° both alone and when mixed with authentic α-spinasterol, and $[\alpha]_D^{17°}$ — 3·7° (c = 1·35, l = 1) (Hart and Heyl, *loc. cit.*, give $[\alpha]_D^{25°}$ — 1·8°).

α-Spinasteryl Acetate.—This was obtained quantitatively from the sterol with acetic anhydride (alone or with pyridine) and crystallised from ethyl acetate in large plates, m. p. 184·5—185·5°. A mixture with authentic α-spinasteryl acetate of identical m. p. gave no depression. It had $[\alpha]_D^{17°}$ — 4·0° (c = 2·32, l = 1), the value of Hart and Heyl (*loc. cit.*) being $[\alpha]_D^{23°}$ — 4·7° (Found: C, 81·6; H, 10·8. Calc. for $C_{30}H_{48}O_2$: C, 81·75; H, 11·0%).

α-Spinasteryl p-Nitrobenzoate.—This was prepared in the usual manner and separated from ethyl acetate–alcohol in fine silky needles which melted at 213·5—215° and gave no depression when mixed with authentic α-spinasteryl *p*-nitrobenzoate of the same m. p. It had $[\alpha]_D^{17°}$ + 5·8° (c = 2·33, l = 1). Hart and Heyl (*loc. cit.*) give $[\alpha]_D^{23°}$ + 4·5°.

α-Spinastadienone.—The sterol (160 mg.) was dissolved in warm 95% acetic acid (60 c.c.) and cooled to 25—30°. The suspension was treated during 10 minutes with chromic anhydride (40 mg.) in glacial acetic acid (10 c.c.) and water (0·3 c.c.) and left at 20° overnight. The clear solution was then concentrated under reduced pressure, and the residue treated with dilute sulphuric acid and extracted with ether. The neutral fraction (140 mg.) on recrystallisation from acetic acid, alcohol, or acetone yielded small plates, m. p. 176—176·5°, which had $[\alpha]_D^{17°}$ + 19·5° (c = 0·77, l = 1) (Found: C, 84·6; H, 11·3. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%).

The *oxime*, prepared in the usual manner, separated from chloroform–methyl alcohol in small plates, m. p. 253—255° (decomp.) (Found: N, 3·9. $C_{28}H_{45}ON$ requires N, 3·4%).

α-Spinasteryl Acetate Oxide.—A solution of α-spinasteryl acetate (460 mg.) in chloroform (10 c.c.) was treated at 0° during 3—4 minutes with a chloroform solution of perbenzoic acid (6·2 c.c.; 1 double bond requires 5·4 c.c.) and left at 5° for 17 hours. The chloroform was removed at room temperature, and the crystalline residue taken up in ether, which was washed with sodium carbonate solution and water and dried. Recrystallisation of the product from acetone gave the pure *oxide* (340 mg.) in parallelograms, m. p. 158·5—159°, $[\alpha]_D^{17°}$ 1·4° (c = 2·67, l = 1) (Found: C, 79·3; H, 10·5. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%).

α-Spinasterol Oxide.—The acetate oxide was refluxed for 2½ hours with 5% methyl-alcoholic potassium hydroxide. The product separated from 90% acetone in prisms, m. p. 164—165° alone or mixed with the *oxide*, m. p. 165°, prepared directly from α-spinasterol and perbenzoic acid (Found: C, 81·4; H, 10·9. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·2%).

Oxidation of α-Spinasteryl Acetate.—To a solution of the acetate (500 mg.) in glacial acetic acid (150 c.c.), chromic anhydride (270 mg.; 3·2 atoms of oxygen) in water (0·5 c.c.) and glacial

acetic acid (10 c.c.) was added in portions during ½ hour at 25°. The solution was left at 20° for 22 hours, methyl alcohol then added, and the solvent removed under reduced pressure. The residue was treated with dilute sulphuric acid, extracted with ether, and separated into acid and neutral fractions with 2% sodium hydroxide solution. The neutral fraction (460 mg.) in alcohol deposited 160 mg. of sparingly soluble, crystalline material, which on repeated crystallisation from absolute alcohol or acetone formed heavy prismatic rods, m. p. 211—213·5° (Found: C, 79·1; H, 10·2. $C_{30}H_{46}O_3$ requires C, 79·2; H, 10·2. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%). An attempt to form an oxime under the ordinary conditions gave a product, m. p. 191—193°, which contained less than 0·5% of nitrogen.

The above acetate was refluxed for 2 hours with 4% alcoholic potassium hydroxide; the solution was then concentrated to half its original volume, and the product precipitated with water and extracted with ether. The residue from the washed, dried, and evaporated extract crystallised from dilute alcohol or acetone in prismatic needles, m. p. 151—152° (Found: C, 81·35; H, 11·0. $C_{28}H_{44}O_2$ requires C, 81·5; H, 10·75. $C_{28}H_{46}O_2$ requires C, 81·1; H, 11·2%).

The alcoholic filtrate from the 160 mg. described above was evaporated, and the residue dissolved in methyl alcohol. The crystalline product which separated was repeatedly crystallised from methanol, small crops of m. p. above 171° being discarded, until the bulk of the material was obtained in soft flattened needles of constant m. p. 170—171°, unchanged by recrystallisation from aqueous acetone (Found: C, 76·6; H, 10·1. $C_{30}H_{46}O_4$ requires C, 76·5; H, 9·9. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·2%).

Action of Bromine on α-Spinasteryl Acetate.—An ice-cold suspension of the acetate (100 mg.) in glacial acetic acid (4 c.c.) and dry ether (8 c.c.) was treated with bromine (40 mg., added in portions during 10 minutes); hydrogen bromide was freely evolved. The now clear solution was diluted with water and extracted with ether, and the extract washed with sodium carbonate solution and water, dried, and evaporated. An alcoholic solution of the semi-crystalline residue yielded unchanged α-spinasteryl acetate (m. p. and mixed m. p. 183°; 50 mg.), the filtrate from which contained only an unstable and highly soluble oil.

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