was obtained. 1-*p*-Bromobenzenesulfon-2,2-diphenylvinylamide dissolved in chloroform reacted rapidly with a chloroform solution of bromine with the evolution of hydrogen bromide.

Summary

1. An investigation has been undertaken to determine whether ethyleneimine derivatives of the type $R_2\underline{C—CH_2—N}R$ can be resolved.

2. Preliminary experiments have indicated that the *p*-bromobenzenesulfonyl derivatives of 1-amino-2-methyl-2-propanol can be converted to the chloride and then to the corresponding ethyleneimine. The replacement of the *p*-bromobenzenesulfonyl group with an optically active sulfonyl group has not yet been successful, since the compounds produced were oils and could not be purified. Corresponding treatment of the *p*-bromobenzenesulfonyl derivative of ethanolamine did not result in the formation of an ethyleneimine.

3. The *p*-bromobenzenesulfonyl derivative of aminomethyldiphenylcarbinol upon treatment with phosphorus pentoxide or thionyl chloride gave 1-*p*-bromobenzenesulfon-2,2-diphenylvinylamide. Similar treatment of 1-*p*-bromobenzenesulfonamido-2-methyl-2-propanol gave what appeared to be a morpholine derivative.

4. Action of sulfuric acid on 1-amino-2-methyl-2-propanol gave *β*-methylallylamine.

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[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

The Isolation of α-Spinasterol from Alfalfa

BY ERHARD FERNHOLZ AND MILDRED L. MOORE

In the course of experiments directed toward the isolation of vitamin K from alfalfa, small amounts of a crystalline sterol were obtained regularly. After a number of recrystallizations it melted at 168°. It was characterized as a sterol by the formation of a digitonide, and by positive Liebermann, Salkowski, and Tortelli-Jaffé reactions. The properties of this sterol suggested to us the possibility that it might be identical with α-spinasterol[1] which was first isolated from spinach,[1] and also has been found in senega root.[2] There is little doubt that the sterol isolated by Dam[3] from alfalfa under similar circumstances and called by him medicagosterol II is in fact α-spinasterol.

The constants observed by us, by Dam, and the latest values for α-spinasterol as recorded by Larsen and Heyl[4] are given in Table I.

TABLE I

	Fernholz and Moore alfalfa sterol		Dam, *et al.* medicagosterol II		Larsen and Heyl α-spinasterol	
	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_D$	M. p., °C.	$[\alpha]_{5461}$
Sterol	168	±0	164	−2.4	172.5	−3.7
Acetate	183	−5	173	...	187	−5.8
m-Dinitrobenzoate	195	±0	195	...	...	...

The data agree well enough if one takes into consideration the difficulties of obtaining the sterol in pure form. The melting point reported for α-spinasterol has gone up steadily in successive publications.

Further evidence for the identity of our sterol with α-spinasterol was obtained by studying its catalytic hydrogenation. Our sterol also took up only one mole of hydrogen using Adams catalyst. The compound obtained was still unsaturated and the melting point and rotation of the dihydrosterol and its acetate agreed well with those reported for α-spinasterol and its acetate. Titrations with perbenzoic acid were carried out, and it was found that 3 atoms of oxygen were consumed by the sterol itself and 2 atoms by the hydrogenated sterol. In the literature, on the other hand, it has been reported[4] that 2 or 1 atom, respectively, is used up. We are unable to explain this difference, but have obtained the same result with authentic α-spinasterol,[5] while stigmasterol under the same conditions consumed a little less oxygen than is calculated for two double bonds. It is not rare, however, that sterols use more perbenzoic acid than the number of double bonds would indicate. A pertinent example is dihydro-

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(1) M. C. Hart and F. W. Heyl, *J. Biol. Chem.*, **95**, 311 (1932).

(2) J. C. E. Simpson, *J. Chem. Soc.*, 730 (1937).

(3) H. Dam, *et al.*, *Helv. Chim. Acta*, **22**, 313 (1939).

(4) Larsen and Heyl, THIS JOURNAL, **56**, 2663 (1934).

(5) We are greatly indebted to Dr. C. Donald Larsen, University of Rochester, for samples of α-spinasterol and its acetate. Mixed melting points with the corresponding derivatives of our sterol did not show a depression.

ergosterol[6] which contains two double bonds and which consumes 2.7 atoms of oxygen. Like α-spinasterol it also contains a double bond resistant to hydrogenation. We do not believe that our results should be interpreted to indicate the presence of three double bonds in α-spinasterol.

We have obtained 0.8 g. of α-spinasterol, m. p. 165°, from 3.8 kg. of alfalfa. Alfalfa meal is probably the most accessible source for this sterol.

Experimental

Isolation of α-Spinasterol.—By percolation with hexane 140 g. of an extract was obtained from 2.8 kg. of dehydrated alfalfa meal. This material was saponified by boiling for two hours with 50 g. of potassium hydroxide in 1 liter of alcohol. The unsaponifiable matter (28 g.) was isolated by ether extraction. It consisted of a dark brown waxy material. It was dissolved in 200 cc. of hot acetone, and the material coming out on cooling was removed by filtration and discarded. The filtrate was evaporated to dryness (20 g.), the residue dissolved in 200 cc. of hexane and washed five times in succession with 200 cc. of 95% methanol saturated with hexane. The combined methanol extracts left on evaporation a residue weighing 11 g. This residue was dissolved in 50 cc. of alcohol. After two days at room temperature the crystallized sterol was filtered and recrystallized from a mixture of alcohol and benzene; yield 0.8 g., m. p. 165°. It was recrystallized nine times more but the melting point could not be raised above 168°; melting point of a mixture with α-spinasterol (169–171°) 168–169°; $[\alpha]^{25}\text{D} \pm 0°$ (21.3 mg. in 2 cc. chloroform, $l = 1$ dm.). *Anal.* Calcd. for $C_{29}H_{48}O \cdot {}^1/_2H_2O$: C, 82.58; H, 11.70. Found: C, 82.70; H, 11.83.

α-Spinasteryl *m*-Dinitrobenzoate.—A sample of 0.1 g. of the above described α-spinasterol was heated on the steam-bath for one hour with 0.3 g. of dinitrobenzoyl chloride and 5 cc. of pyridine. The dinitrobenzoate was then isolated by means of ether and purified by crystallization from benzene by addition of alcohol as small white needles, m. p. 195°, $[\alpha]\text{D} \pm 0°$ (27.2 mg. in 2 cc. of chloroform, $l = 1$ dm.). *Anal.* Calcd. for $C_{36}H_{50}O_6N_2$: C, 71.24; H, 8.31. Found: C, 71.11; H, 8.39.

α-Spinasteryl acetate was prepared from the sterol isolated from alfalfa by refluxing with ten times the amount of acetic anhydride for one hour. The acetate crystallized out after cooling and was recrystallized from benzene by addition of alcohol, in which solvent it is but sparingly soluble; m. p. 183°, mixed with authentic material (184.5°) also 183°; $[\alpha]^{23}\text{D} - 5.3°$ (33.8 mg. in 2 cc. chloroform, $l = 1$ dm., $\alpha\text{D} - 0.09°$). *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.87; H, 11.09. Found: C, 81.74; H, 11.02.

α-Spinastenyl Acetate.—The acetate described above (0.5163 g.) was hydrogenated in acetic acid solution (50 cc.) at room temperature and ordinary pressure with a platinum oxide (0.1643 g.) the hydrogen consumption of which was known from a blank determination. The calculated amount of hydrogen (49.6 cc.) was absorbed within eighty minutes. The hydrogenation product was recrystallized from a benzene–alcohol mixture as large leaflets, m. p. 117°, $[\alpha]^{24}\text{D} + 15.3°$ (20.9 mg. in 2 cc. chloroform, $\alpha\text{D} + 0.16°$]. *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.58; H, 11.48. Found: C, 81.80; H, 11.74. The literature[4] reports m. p. 119°, $[\alpha]_{5461} + 12°$.

α-Spinastenol was obtained from the acetate by saponification with 5% alcoholic potassium hydroxide. It was purified by crystallization from methanol and obtained in the form of big leaflets. An air-dried sample melted on slow heating at 110°, a value also reported in the literature; immersion into a bath at 100° caused melting at that temperature. The compound thoroughly dried in a high vacuum at 80° melts at 112–114°. At times the compound crystallized in the form of small crystals of this melting point without having been dried at 80°; $[\alpha]^{24}\text{D} + 26°$ (26.5 mg. in 2 cc. of chloroform, $l = 1$ dm., $\alpha\text{D} + 0.34$). *Anal.* Calcd. for $C_{29}H_{50}O$: C, 83.98; H, 12.16. Found: C, 83.68; H, 12.35.

Titration with Perbenzoic Acid.—The chloroform solution of perbenzoic acid used in these determinations was several months old and contained approximately 3 mg. of active O per cc. The substance was dissolved in 2 cc. of chloroform and 2 cc. of the perbenzoic acid solution added. Blanks were made with the same amount of chloroform and perbenzoic acid. After standing at 0° for three days the samples were titrated and the consumption of oxygen determined by difference. α-Spinasterol from alfalfa: 41.3 mg. consumed 4.57 mg. O = 2.95 atoms; α-spinasterol from spinach: 31.5 mg. consumed 3.6 mg. O = 3.05 atoms; α-spinastenyl acetate: 25.8 mg. consumed 1.97 mg. O = 2.18 atoms; stigmasteryl acetate: 21.2 mg. consumed 1.38 mg. O = 1.81 atoms.

Summary

The isolation of a sterol from alfalfa has been described together with evidence showing its identity with α-spinasterol.

It has been pointed out that Dam's medicagosterol II also obtained from alfalfa is in all probability α-spinasterol.

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[6] Windaus and Lüttringhaus, *Ann.*, **481**, 120 (1930).