[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, THE UPJOHN COMPANY]

The Unsaponifiable Fraction of Spinach Fat. A Glucoside of γ-Spinasterol

BY FREDERICK W. HEYL AND DONALD LARSEN

In working up a large quantity of spinach for the purpose of further work on the sterols we have altered the process, and, after the isolation of the fats, have extracted the fat-free resin with ether and alcohol, and from this fraction a highly crystalline new phytosterolin separated in quantity. Inasmuch as the fats have been removed and, further, since the glucoside itself could be crystallized from pyridine–alcohol, it was possible upon hydrolysis to isolate the sterol without the burdensome fractional crystallization which must be employed in working with the saponified lipoid mixture.

The new sterol, γ-spinasterol, proved to be optically inactive. We have described some of the corresponding esters.

This sterol appears to be an isomer of α-spinasterol[1] and of β-spinasterol.[2] On reduction the same dihydro derivative results.

In previous publications we ascribed to α- and β-spinasterol the empirical formula corresponding to C_{27}. Since that time the formulas of several of the sterols have been revised to C_{28}.[3]

In a number of quantitative saponifications, which we carried out on some of the esters of γ-spinasterol, we have obtained data which lead us to believe that the empirical formula for these spinach sterols should be revised to correspond to C_{28}.

Experimental

Four hundred seventeen pounds of dry spinach were percolated with 95% alcohol. The alcoholic extract was precipitated with water, filtered, and the precipitated resin mixed with purified sawdust, which had been thoroughly extracted with organic solvents. This resin–sawdust mixture was extracted with petroleum ether (30–60°) for one hundred hours. This contains the lipoid material. The sawdust was then percolated with ether for sixty hours, which removed most of the pigment and a small amount of phytosterolin. The resin–sawdust was then extracted eight times with large volumes of boiling 95% alcohol. After standing several days these combined alcoholic extracts yielded 40 g. of an amorphous phytosterolin pigment precipitate. Digestion of the latter with petroleum ether and ether removed 5 g. of pigment. Digestion of the residue with hot chloroform–alcohol and repeated crystallizations from pyridine–alcohol (1:1) yielded about 14 g. of white crystalline glucoside. About 1 g. of the same phytosterolin was isolated from the ether percolate. Recrystallizing the glucoside six times from pyridine–alcohol gave m. p. 275–280° with decomposition; $[\alpha]^{22}_{5461}$ $-33.0°$.

Tetraacetyl Ester.—This ester was made by boiling the glucoside in acetic anhydride. It was recrystallized from alcohol, giving fine needles, m. p. 178.5–179°, $[\alpha]^{20}_{5461}$ $-13.4°$, in chloroform.

Tetrabenzoyl Ester.—This ester was made in the usual manner with benzoyl chloride, using pyridine as solvent. The ester crystallized from alcohol in hexagonal plates, melting at 178–178.5°, $[\alpha]^{20}_{5461}$ $+21.6°$, in chloroform.

Analytical data, molecular weights, etc., of the phytosterolin and esters are listed below (Table I, 1–3).

Hydrolysis of the Glucoside.—The sterol was hydrolyzed by the method of Power and Solway.[4] The aqueous filtrate, containing the sugar, showed reducing sugars with Fehling's solution. Upon treatment with phenylhydrazine hydrochloride an osazone was formed having the characteristic needle grouping of glucosazone. Recrystallized from alcohol–water (1:1), m. p. 207–208°.

Examination of the Sterol Fraction: γ-Spinasterol.—The water-insoluble portion of the hydrolysis mixture yielded approximately 10 g. of homogeneous, optically inactive sterol melting at 159–160°. The mother liquors contained a small amount of very low melting material, possibly degradation products, which has not been investigated.

Several esters of this γ-spinasterol were prepared. They are listed below with their analyses, melting points, specific rotations, etc. (Table I, 4–8). The molecular weights were determined by parallel saponifications with blanks of 2% alcoholic potassium hydroxide, and titrating the excess with standardized *N*/10 hydrochloric acid.

Comparison of γ-Spinasterol with α-Spinasterol.—Three aliquots of 0.021 g. each of γ-spinasterol acetate in glacial acetic acid were subjected to micro-catalytic hydrogenation with Adams[5] catalyst. Data for 0.93, 1.05 and 1.07 double bonds were obtained. 0.5 g. of the acetate was reduced on a macro scale. The combined dihydro solutions, after boiling in acetic anhydride, yielded the acetate in colorless flat plates. Analyses and other physical constants appear in Table I. A mixed melting point, with dihydrospinasterol acetate prepared from α-spinasterol showed no depression; m. p. 116–117°.

The above acetates, obtained from two different sterols, when saponified, gave dihydrosterols which appear to be identical. From α-: m. p. 106–107°; from γ-: m. p. 105–106°; mixed m. p. 106–107°. These data lead one to believe that the α- and γ-forms are isomers.

0.2 g. of the dihydrospinasterol was subjected to the modified Liebermann–Burchard reaction of Anderson and

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

(2) F. W. Heyl and D. Larsen, *J. Am. Pharm. Assoc.*, **22**, 510 (1933).

(3) A. Windaus and A. Lüttringhaus, *Nach. Ges. Wiss. Göttingen*, 4 (1932); A. Windaus, F. v. Werder and B. Gschaider, *Ber.*, **65**, 1006 (1932); I. M. Heilbron and J. C. E. Simpson, *J. Chem. Soc.*, 2400 (1932).

(4) F. B. Power and A. H. Solway, *J. Chem. Soc.*, **103**, 399 (1913).

(5) "Organic Syntheses," Vol. VIII, p. 92.

COMPOUNDS, PROPERTIES AND ANALYSES

All samples for micro-analyses were dried in a high vacuum at temperatures slightly below the melting points of the respective compounds

				Calculated, %				Found, %				Recovered compound	
Compound	M. p., °C.	$[\alpha]_{5461}$[a]	Formula	Mol. wt.	C	H	N	Mol. wt.	C	H	N	M. p., °C.	$[\alpha]_{5461}$
Phytosterolin	275–288	−33.0	$C_{34}H_{56}O_6$	560	72.8	10.2			72.7	10.4			
Tetraacetate	178.5–179	−13.4	$C_{42}H_{64}O_{10}$	728	69.2	8.9		721 735	69.2	9.2		275–280	−33.0
Tetrabenzoate	178–178.5	+21.6	$C_{62}H_{72}O_{10}$	977	76.2	7.5		960 972	75.9	7.6		275–280	−33.0
γ-Spinasterol	159.5–160	0	$C_{28}H_{46}O$	398	84.3	11.6			84.3	11.5			
γ-Acetate	139.5–140	−14.1	$C_{30}H_{48}O_2$	440	81.7	11.0		441 437 439 440	81.65	11.1		159–159.5	0
γ-Benzoate	118.5–119	−10.3	$C_{35}H_{50}O_2$	502	83.6	10.0		517 525	83.6	10.3		158–159	0
γ-*p*-Nitrobenzoate	200	− 8.9	$C_{35}H_{49}O_4N$	547	76.7	9.0	2.6	554 552 547 555	76.7	8.9	2.7	158–159	0
γ-Phenylurethan	144–145	−15.9	$C_{35}H_{51}O_2N$	517	81.1	9.9	2.7		80.9	10.2	2.9	158–159	0
Dihydro-γ-spinasterol	105.5–106	+24.0	$C_{26}H_{48}O$	400	83.9	12.1			83.9	12.1			
Dihydro-γ-acetate	115.5–116	+12.4	$C_{30}H_{50}O_2$	442	81.4	11.4		444 446	81.2	11.6		105.5–106	+24.0

[a] All rotations were taken with chloroform as solvent, except 1, in which pyridine was used.

Nabenhauer.[6] If this reaction may be considered a method of separating plant sterols into saturated and unsaturated groups, it would then appear that dihydrospinasterol still contains one or more double bonds, for we were unable to detect any sterol material in the carbon tetrachloride layer, when the conditions of the above reaction were employed. Perhaps the inactive double bond resembles the inactive double bond of α-ergostenol.[7] This problem is now under investigation.

Summary

1. A new phytosterolin has been described.
2. Hydrolysis of the phytosterolin yields optically inactive γ-spinasterol.
3. γ-Spinasterol probably contains two double bonds and appears to be an isomer of α-spinasterol.
4. A number of esters of γ-spinasterol have been prepared, as well as dihydrospinasterol and its acetate.

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[6] R. J. Anderson and F. P. Nabenhauer, THIS JOURNAL, **46**, 1959 (1924).

[7] M. C. Hart and H. Emerson, *ibid.*, **54**, 1070 (1932); I. M. Heilbron and D. G Wilkinson, *J. Chem. Soc.*, 1708 (1932).

[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY OF THE UNIVERSITY OF CHICAGO]

Studies of Conjugated Systems. X. The Electrolytic Reduction of Vinylacrylic Acid

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In a recent paper[1] on the catalytic hydrogenation of compounds containing conjugated systems of double bonds, we presented a classification of the various types of reducing agents based on their effect on ethylenic double bonds. The classification was as follows.

(1) This group includes the soluble reducing agents, such as ferrous chloride, vanadous chloride, chromous chloride, stannous chloride and titanous chloride. These reagents are known to reduce the carbonyl group, C=O, but are without action on ethylene hydrocarbons.

(2) A second group includes the amalgams of the alkali metals and those of aluminum and magnesium. In this group also belong such reducing agents as the alkali metals and their alcoholates, zinc and hydrochloric acid, and similar reducing agents. The reagents of this group do reduce ethylene double bonds, and will usually reduce a conjugated system in the terminal positions.

(3) Catalytic methods may also be used to hydrogenate ethylene double bonds, but in contrast to the action of the reagents of group (2), reduction of conjugated systems by catalytic means does not occur in the 1,4-positions.

No work has been reported on the electrolytic reduction of compounds containing conjugated systems of double bonds of the type C=C—C=C, but it was stated in our previous publication that electrolytic reduction would probably fall in the second group. The present paper deals with the electrolytic reduction of vinylacrylic acid and shows that it properly belongs in the second group.

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[1] Muskat and Knapp, *Ber.*, **64**, 779 (1931).