

ometers were used so the melting points may be regarded as corrected. The molecular weights were determined by titrating the esters, dissolved in 50% alcohol, with standard sodium hydroxide. The yields averaged from 33 to 50%.

Discussion

Due to the ease of hydrolysis and the time necessary at low temperature for crystallization, the ethyl ester was not isolated in pure enough form for analysis, hence Amann's value is used in the curve. The esters are white crystalline solids, having very definite melting points and soluble in organic solvents. There is sufficient variation in the melting points through dodecyl so that they could be used very satisfactorily as checks on the identification of the individual alcohols. It will be noted that up to decyl the melting points give a curious pattern and from this ester on, alter-

nation gradually increases, both the odd and even ones forming smooth curves.

This problem was suggested by Dr. E. Emmet Reid, Research Adviser, University of South Carolina, and to him the authors wish to express their appreciation for assistance, and for the higher alcohols, the odd ones from undecyl to heptadecyl being those prepared by Meyer and Reid.²

Summary

The monoalkyl phthalates of the normal alcohols from methyl to eicosyl have been prepared and their melting points determined. A melting point curve is shown; it exhibits regular alternation above the decyl ester.

(2) Meyer and Reid, *THIS JOURNAL*, **55**, 1574 (1933).

COLUMBIA, S. C.

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[CONTRIBUTION FROM THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE OF AGRICULTURE AND APPLIED SCIENCE]

The Sterols of Alfalfa Seed Oil. I

BY L. CARROLL KING AND CHARLES D. BALL, JR.

1. Introduction

The fat constants and fatty acids of alfalfa seed oil have been studied by Jacobson and Holmes,¹ Schuette, Vogel and Wartinbee,² and by Schuette and Vogel.³ As far as can be determined, there has been no previous account of an investigation of the unsaponifiable material from alfalfa seed oil.

About 4% of the oil which can be extracted from alfalfa seed with ethyl ether is unsaponifiable. This unsaponifiable portion consists of a crystalline substance or substances which represent about 35% of the total unsaponifiable, and of a clear, reddish, oily liquid which makes up the remainder.

The crystalline material consists primarily of a sterol very similar to or identical with the sterol isolated by Heyl, Wise and Speer⁴ and Clenshaw and Smedley-MacLean⁵ from spinach fat and later termed α -spinasterol by Hart and Heyl.⁶ Simpson⁷ in 1937 isolated α -spinasterol from senega root. The formula first assigned to α -

spinasterol by Hart and Heyl⁶ was $C_{27}H_{46}O$. Recent work by Larsen⁸ on the completely reduced sterol, spinastanol, showed the latter to be identical with fucostanol and stigmastanol, for which the formula $C_{29}H_{52}O$ has been accepted. Since Larsen and Heyl⁹ showed α -spinasterol to be doubly unsaturated, the formula for α -spinasterol, therefore, would be $C_{29}H_{48}O$.

The reddish oily fraction gives a positive Liebermann-Burchard reaction, but colors only the sulfuric acid layer in the Salkowski reaction. The Whitby B reaction¹⁰ is positive. Further work on this material is in progress in this Laboratory.

2. Experimental

Extraction of Seed.—Ground alfalfa seed (*Medicago sativa* L.) was extracted continuously with hot diethyl ether. The extract was then filtered and the solvent distilled off in the presence of an atmosphere of carbon dioxide; yield 11.8% of a greenish oil which had an index of refraction of 1.4820 at 15°. The fat constants of this oil were in good agreement with those obtained by Jacobson and Holmes.¹

Saponification.—To 100 g. of alfalfa seed oil was added 600 ml. of ethyl alcohol and 100 ml. of 50% potassium hydroxide. This mixture was refluxed for one hour,

(1) Jacobson and Holmes, *THIS JOURNAL*, **38**, 480 (1916).

(2) Schuette, Vogel and Wartinbee, *Oil and Soap*, **15**, 35 (1938).

(3) Schuette and Vogel, *ibid.*, **16**, 16 (1939).

(4) Heyl, Wise and Speer, *J. Biol. Chem.*, **82**, 111 (1929).

(5) Clenshaw and Smedley-MacLean, *Biochem. J.*, **23**, 107 (1929).

(6) Hart and Heyl, *J. Biol. Chem.*, **95**, 311 (1932).

(7) Simpson, *J. Chem. Soc.*, 730 (1937).

(8) Larsen, *THIS JOURNAL*, **60**, 2431 (1938).

(9) Larsen and Heyl, *ibid.*, **56**, 2663 (1934).

(10) Whitby, *Biochem. J.*, **17**, 5 (1928).

cooled and exhaustively extracted with petroleum ether. The petroleum ether extracts were washed free from potassium hydroxide, using 10% alcohol. The solvent was then evaporated; yield 4 g. of unsaponifiable material.

Separation of Fractions.—The unsaponifiable fraction was taken up in 50 ml. of petroleum ether and allowed to stand. A slow growth of crystalline material took place. After one week this crystalline material was centrifuged, washed twice with petroleum ether, and recrystallized, once from petroleum ether and twice from absolute alcohol. The resulting crystals (Fraction I) were cholesterol-like plates and had a melting point of 164.4–164.8°. The mother liquor from Fraction I was reduced in volume to 25 ml. and allowed to stand. A second crop of crystals identical with Fraction I was obtained.

The mother liquor from the above fraction was placed in the icebox. A slow growth of crystalline material again took place. After two weeks this crystalline substance was separated. It was recrystallized once from petroleum ether and twice from absolute alcohol. The crystals (Fraction II) were elongated hexagonal plate-like structures, and had a melting point of 160–161°.

The mother liquor from the above fractions was evaporated to about 10 ml. and returned to the icebox for three weeks. The crystalline material, which grew slowly, was separated by diluting with cold petroleum ether and centrifuging. The substance was recrystallized once from petroleum ether and three times from absolute alcohol. The crystals (Fraction III) were elongated hexagonal plate-like structures and melted at 155–156°. No further crystalline material could be separated from the mother liquors. The reddish oil remaining weighed 2.6 g. and represented about 65% of the total unsaponifiable fraction.

Characterization of Fraction I.—The Liebermann-Burchard and Whitby B reactions were positive; the Salkowski reaction, negative. A series of esters of Fraction I was prepared. To prepare the acetate the sterol was refluxed in acetyl chloride until solution was effected, and for about ten minutes thereafter. The reaction mixture was cooled and then poured into water. The ester was then filtered off, washed with water and recrystallized twice from alcohol. The benzoyl ester, the *p*-nitrobenzoyl ester and the butyryl ester were prepared according to the procedure given by Hart and Heyl,⁶ except that they were recrystallized from absolute alcohol. The phenylurethan was prepared by treating the sterol with phenyl isocyanate in petroleum ether solution. The product was recrystallized twice from absolute alcohol and once from ethyl acetate. Carbon and hydrogen determinations were run on the acetyl, the benzoyl and the *p*-nitrobenzoyl esters by the micro methods of F. Pregl.¹¹

A summary of the constants and analyses of these compounds is given in Table I. The melting points of these derivatives checked quite closely with the corresponding derivatives for α -spinasterol cited by Sobotka.¹²

Bromination of Fraction I.—The sterol when dissolved in carbon disulfide and a solution of bromine in carbon disulfide added, gave only a soluble oil. Attempts to bromi-

(11) Roth, translated by Daw, "Quantitative Organic Micro-analysis of F. Pregl," P. Blakiston's Son and Co., Inc., Philadelphia, Pa., 1937.

(12) Sobotka, "Chemistry of the Sterids," The Williams and Wilkins Co., Baltimore, Md., 1938.

TABLE I

Compound	M. p., °C.	Analysis, %			
		C		H	
		Calcd. ^a	Found	Calcd. ^a	Found
Sterol	164.4–164.8	84.4	83.0	11.7	11.7
Sterol acetate	180–181	81.93	81.84	11.01	11.21
Sterol benzoate	196–197	83.72	83.52	10.08	10.44
Sterol <i>p</i> -nitrobenzoate	211–213	77.00	76.34	9.26	9.49
Sterol phenylurethan	173–174				
Sterol butyrate	123–125				

^a The required values are calculated from the formula $C_{29}H_{48}O$.

nate in ether gave the same result. An attempt to brominate the acetate in ether solution also resulted in the formation of a soluble oil.

Mixed Melting Point Determinations.—A mixed melting point determination on Fraction I and α -spinasterol¹³ gave no depression. The acetyl and *p*-nitrobenzoyl esters of α -spinasterol were prepared according to the same procedure used in preparing the corresponding esters of Fraction I. These compounds had the same melting points as the respective esters of Fraction I, and when corresponding esters from each source were mixed there was no melting point depression.

Preliminary work on Fractions II and III indicated that the crystalline substance in these fractions is also mainly α -spinasterol. However, the melting points of the derivatives corresponding to those for Fraction I were uniformly lower. Mixed melting point data indicated that other material contaminated the sterol. The Liebermann-Burchard, Whitby B, and Salkowski reactions were practically identical with those obtained for Fraction I. Further work on these fractions is in progress.

3. Discussion

A comparison of the melting point data, mixed melting point data, and analysis of the alfalfa seed sterol with that for the sterol reported by Heyl, Wise and Speer,⁴ and later termed α -spinasterol by Hart and Heyl,⁶ and by Larsen and Heyl⁹ indicate that the alfalfa seed sterol is identical or very closely related to α -spinasterol. The observed and calculated values for the percentage of carbon in the free sterol leave something to be desired. However, it is interesting to note that if the data presented by Heyl, Wise and Speer⁴ be calculated in the same manner as in the present work and not on the assumption of the presence of 1.0 molecule of water, their values for the percentage of carbon and percentage of hydrogen would be 83.2 and 11.7, respectively, agreeing closely with those reported in this paper. Further, the alfalfa seed sterol failed to give a crystalline bromide. The same observation was made

(13) We are indebted to Dr. Koloff of the Upjohn Research Laboratories who very kindly supplied us with a specimen of α -spinasterol.

by Larsen and Heyl⁹ and by Simpson⁷ when they attempted to brominate α -spinasterol.

Summary

1. The unsaponifiable fraction of alfalfa seed

oil has been separated into a crystalline substance and an oily mixture.

2. The crystalline substance is believed to be α -spinasterol.

EAST LANSING, MICHIGAN

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Porphyrin Studies. III.¹ The Structure of the Porphine² Ring System

BY PAUL ROTHEMUND

The importance of the porphine ring system is evident from the fact that it is the fundamental ring system of all the porphyrins. "Chlorins" are dihydroporphines, and the—thus far hypothetical—"Phorbine" ring, which is present in the chlorophylls, differs from chlorin only by the addition of an isocyclic ring in δ, γ -position.

Some time ago the author reported the synthesis of porphine by the interaction of pyrrole and formaldehyde.³ It was found that many aldehydes can be condensed with pyrrole to yield porphines, in which the four *meso* (*ms*-) positions α , β , γ , and δ , carry the residue from the corresponding aldehyde as substituent. The reaction seems to be a general one of aldehydes, for more than 25 aliphatic, aromatic, and heterocyclic aldehydes gave positive results in this porphyrin formation. In this synthetic work a number of facts relating to the structure of the porphine ring have been observed.

In the second publication quoted above³ it was mentioned that under certain conditions the reaction mixture from the porphine synthesis contained, in addition to porphine, a porphyrin with a hydrochloric acid number much lower than that of porphine. In comparison with porphine the two main absorption bands of this porphyrin were shifted about 100 Å. toward the red region of the spectrum. The new porphyrin formed only in small quantities, while porphine of hydrochloric acid number 3.3 was the main product.

(1) Paper II, THIS JOURNAL, **58**, 625 (1936).

(2) In preceding papers we spelled "Porphin," because the term was introduced in this form into the chemical literature by H. Fischer for the—at that time hypothetical—fundamental ring system of porphyrins [H. Fischer and P. Halbig, *Ann.*, **448**, 193 (1926)]. Dr. Austin M. Patterson, member for the United States of the Committee on Organic Nomenclature of the International Union of Chemistry, and Chairman of the American Committee, recommended strongly to change the spelling in English to "Porphine," the final *e* signifying the basic character of the compound. He recommended further that the spelling of porphyrin be maintained.

(3) Rothemund, THIS JOURNAL, **57**, 2010 (1935); **58**, 625 (1936).

The hydrochloric acid number was 0.5, and the elementary analysis checked with that of porphine within the limits of analytical error. Each of the two porphyrins formed complex metal salts with magnesium ("phyllin"), iron ("hemin"), and copper. The low yields of the porphyrin with hydrochloric acid number 0.5 have prevented the preparation of complex metal salts other than the ones mentioned above so far, but from the component of hydrochloric acid number 3.3 the silver, zinc, cadmium, cobalt and nickel salt were prepared.

The direct determination of the molecular weight of the two porphyrins met with failure so far, but from analytical and spectroscopic data of the free compounds and of their metal complex salts, the author assumes that the new porphyrin isolated in the synthesis of porphine from pyrrole and formaldehyde is the isomer of porphine, and he suggests the term "isoporphine" for it. Two structural formulas may represent the difference in structure of the two compounds: formula I with hydrogen atoms linked to the nitrogen atoms in rings I and II (adjacent rings), and formula II, in which these hydrogen atoms are attached to nitrogen in rings II and IV (opposite rings).

There is, at present, no possibility of assigning one configuration absolutely to either compound, hence the use of the name porphine for structure I, and isoporphine for structure II is still arbitrary.

These structural formulas are drawn to show a double bond between carbon atom γ and the α carbon atom of ring IV (nomenclature of H. Fischer⁴). The presence of a double, not a single, bond between these carbon atoms has been postulated from physicochemical data by Stern.⁵

(4) Fischer, Halbig and Walach, *Ann.*, **452**, 268 (1927); Fischer, Moldenhauer and Süs, *ibid.*, **486**, 107 (1931).

(5) Stern and Wenderlein, *Z. physik. Chem.*, **A175**, 405 (1936).