

(c) *Reduction with Hydrogen Sulphide.* By treating dibromthymoquinone with an ammoniacal solution of H_2S , a small crop of yellow crystals, m. p. 118–119° was formed, which as yet have not been investigated any further.

REFERENCES.

- (1) The detailed results of this physiological study have been left for a future paper.
- (2) Schniter, *Ber.*, 15 (1882), 657.
- (3) Carstanjen, *J. prakt. Chem.*, 111 (1871), 50.
- (4) Checkik, "From Cymene to Dihydroxythymoquinone," unpublished thesis, U. W., 1931.
- (5) Kehrman, *Ber.*, 22 (1889), 3264.
- (6) Schulz, *Ibid.*, 15 (1882), 657.
- (7) Schniter, *Ibid.*, 20 (1887), 1318.
- (8) J. Schmidt, "A Textbook of Organic Chemistry" (1926), page 356.
- (9) Schulz, *loc. cit.*, page 658.

WISCONSIN PHARMACEUTICAL EXPERIMENT STATION.

THE UNSAPONIFIABLE FRACTION FROM SPINACH FAT. II.

BY FREDERICK W. HEYL AND DONALD LARSEN.

In a previous publication (1) from this laboratory, the isolation of a hydrocarbon melting at 67.5–68.5° and two alcohols melting at 76–77° and 87–88°, respectively, was described. The second of these alcohols, of which we obtained but 0.2 Gm., appeared to be a glycol.

In recent papers (2) (3) it has been shown that the hydrocarbon is $C_{31}H_{64}$, hentriacontane.

The point was brought up by the English workers concerning the identity of the alcohol melting at 76–77° with ceryl alcohol (C, 81.67; H, 14.13) with which conclusion our previously reported results are at variance (C, 80.9; H, 14.2). The spinach which we extracted presents a highly complex mixture. It contained not only a preponderant amount of hentriacontane but also a glycol and the last substance appears to be absent from English spinach. The glycol for which we previously reported a m. p. of 87–88° has now been obtained purer and melts at 103.5°.

Elaborate effort has been made to definitely describe the monohydric alcohol and to identify it with certainty. This problem is greatly facilitated by the recent work of Pollard, Chibnall and Piper (4) who isolated pure *n*-hexacosanol from cocksfoot grass (*Dactylis Glomerata*). They reported that the alcohol melts at 79.7° and its acetate at 59.5–59.6°. Collison and Smedley-Maclean found for their ceryl alcohol from spinach 77–78° and for the acetate 61.5°.

Our further work shows conclusively that the spinach alcohols are a mixture of *n*-tetracosanol and *n*-hexacosanol with the former predominating. It is also shown that fractionation of the paraffin alcohols or of their acetates does not give the satisfactory analysis of this fraction which results finally from working with the corresponding acid mixtures.

Incidentally a new dextro-rotatory sterol has been isolated from the mother liquors of the less soluble spinasterol.

EXPERIMENTAL.

The unsaponifiable material was crystallized from large volumes of 95 per cent alcohol (50 Gm. in 2.5 liters) and the crystals were filtered off after a few hours' standing. These were worked up as reported in the work on spinasterol (5).

The combined filtrates were fractionated and the more insoluble fractions, below this sterol, were digested with relatively small amounts of ether, whereupon an insoluble glycol melting at 103.5° was isolated. (The probable formula is $C_{26}H_{54}O_2$.)

Our work concerns the soluble material in the filtrate. The ether was removed and a prolonged fractionation from methyl alcohol gave a top fraction weighing 5.0 Gm. which was largely hydrocarbon (m. p., 67–72°). Successive crops below this melted, respectively, 70–80°; 65–105°; 70–120°; 65–110°, and a final filtrate (A).

While this work was in progress, we also carried out work upon the alcohol-insoluble material designated (*a*) in our first paper (1). We crystallized this material from alcohol and obtained a top fraction of hydrocarbon and then we repeatedly recrystallized the more soluble material from ether. The melting point of the purified material rises slowly from 73° to 76–77°. Other solvents such as ligroin, chloroform, acetone or methyl alcohol yield no purer crystalline material. This substance corresponds exactly with that previously described. Acetylation gives an acetate melting at 58–59°. The acetate of this fraction obtained by Collison and Smedley-Maclean (3) melted at 61.5°.

Analysis gave figures checking very closely for $C_{22}H_{45}O.CO.CH_3$. (Calc., C, 78.17; H, 13.13. Found, C, 78.13; H, 12.9.) However, when saponified a mean molecular weight of 432 was obtained (calc., 368). The free alcohol recovered after saponification was converted into the acid phthalate by the method of Power (6). Recrystallized four times the acid phthalate melted at 80–81°. The alcohol recovered upon saponification melted at 78–79°. The corresponding acetate melted at 59.5–60.5°. (C, 80.1; H, 13.4.)

It appears that a more systematic study of this mixture is necessary as traces of hydrocarbon and other possible contaminants cause a continued lack of agreement between the percentages of C and H found and the expected mean molecular weight.

We, therefore, took the filtrate (A) and the corresponding filtrate from the work on the acetate just described and precipitated the soluble sterol with digitonin. In the case of the preparation of the acetate melting at 58–59°, repeated recrystallizations from ether remove this sterol from the top fractions.

After filtering the insoluble digitonide, the excess of digitonin was removed from the filtrate which was thereupon evaporated to dryness. The residue was separated into (*a*) the alcohol fraction and (*b*) the hydrocarbon fraction by means of the phthalic acid fusion method.

The Dextro-rotatory Sterol.—The sterol fraction was liberated from the digitonide by xylene and then fractionated from absolute alcohol. Less soluble spinasterol rich fractions were discarded and the more soluble fractions consisting largely of the new dextro-rotatory sterol gave 7 successive crops. $(\alpha)_{5461} + 5.77^\circ$ to 6.7° . M. p., 143–149°. Further purification could not be accomplished by this process

and the material was acetylated and the acetate exhaustively and systematically crystallized from ether and alcohol and a further quantity of spinasterol rich sterol separated as acetate $(\alpha)_{5461} = -1.16^\circ$. From the filtrates, after the exclusion of several intermediate crops, acetates were isolated in a series showing $(\alpha)_{5461} = +6.3^\circ$ to 6.8° .

The purest material melted at $150-154^\circ$ and $(\alpha)_{5461} = +7.2^\circ$.

$C_{29}H_{48}O_2$. Calcd., C, 81.24; H, 11.29
Found, C, 81.23; H, 11.46.

The acetate was saponified by boiling for 15 minutes with 3 per cent alcoholic potash whereupon the sterol separated in plates. Recrystallized from alcohol + ether, it melted at $145-148^\circ$ and $(\alpha)_{5461} = 7.65^\circ$. No insoluble bromide could be prepared.

The Liebermann-Burchard and Salkowski color tests were positive; the trichloroacetate test of Rosenheim was negative.

The sterol acetate on quantitative reduction in acetic acid solution with platinum oxide as a catalyst absorbed hydrogen approximately for one double bond. The reduced sterol acetate after crystallization from acetic acid gave flat plates melting at $112-113^\circ$. A mixed melting point with spinastanol acetate, prepared by the catalytic reduction of spinasterol acetate, was not lowered. β -spinasterol, the more soluble sterol from spinach, is therefore closely related to spinasterol giving the same spinastanol on catalytic reduction.

The fraction consisting of paraffin alcohols was acetylated and the acetates were extensively fractionated from large volumes of absolute alcohol and collected in six fractions: 1, m. p., $62.5-64^\circ$; 2, $61.5-63.5^\circ$; 3, $60-62^\circ$; 4, $58-60^\circ$; 5, $56.5-58.5^\circ$; 6, $52.5-55^\circ$. The molecular weights found upon saponification varied from 467 to 397, but since all fractions were amorphous no more reliable interpretation was possible here than in the case of the acetate previously described.

The alcohols recovered upon saponification from the above six fractions were studied with the following results:

TABLE I.—THE ALCOHOL FRACTIONS.

Fraction No.	Weight.	M. p.	M. p.*	C.	H.
1	0.025	79-81	...	81.98	14.22
2	0.26	75-79	79.5-81	81.75	14.37
3	0.82	79.81	80-81	81.57	14.03
4	0.73	76-79	77-78	81.12	14.07
5	2.12	75-78	76-77	80.89	13.95
6	0.20	77-79	78-79	80.58	13.97
			Calc. $C_{30}H_{62}O$	82.10	14.25
			$C_{28}H_{58}O$	81.86	14.24
			$C_{26}H_{54}O$	81.58	14.23
			$C_{24}H_{50}O$	81.26	14.22
			$C_{22}H_{46}O$	80.89	14.21

* The melting points in the first column are those found after once recrystallizing from alcohol; the second column represents the material after a number of further crystallizations from ether; Fraction 3, after 3; Fraction 5, after 5 more crystallizations.

Since the work on the alcohols agreed in every respect with our work on the acetates, a conclusion might be justified that we had here a complicated mixture of

a series of alcohols including C_{30} to C_{22} members. But the results of oxidation did not bear this out.

Each fraction was now oxidized by the method of Gascard (7).

Fraction 2 gave a product which upon crystallization from alcohol had a m. p. of $82-83^\circ$. C, 78.56; H, 13.5. It was recrystallized from alcohol twice, alcohol and ether twice, acetone twice and again from alcohol and ether. M. p., $83-83.5^\circ$. C, 78.3; H, 13.24.

Calc. for $C_{26}H_{52}O_2$. C, 78.71; H, 13.22.

$C_{24}H_{48}O_2$. C, 78.17; H, 13.13.

Fraction 3 which was a highly crystalline alcohol gave an acid, m. p., $83-85^\circ$. C, 78.58; H, 13.05. Recrystallized eleven times, m. p., $87-87.5^\circ$. C, 78.68; H, 13.12. This is pure hexacosanic acid and the corresponding alcohol is pure *n*-hexacosanol. Pollard, Chibnall and Piper (4) found the melting point of the pure acid $87.7-87.9^\circ$ and for the *n*-hexacosanol 79.7° ; for the acetate $59.5-59.6^\circ$.

Fraction 4 gave an acid which upon one crystallization had a m. p. of $78-80^\circ$. C, 78.14; H, 13.23. Recrystallized eight times from alcohol and ether, m. p., $81-82^\circ$. C, 78.53; H, 13.51. This is a mixture of the C_{24} and C_{26} acids.

Fraction 5 which is a crystalline alcohol when oxidized gave an acid, m. p., $80-82^\circ$. C, 78.22; H, 12.87. Recrystallized from alcohol and from alcohol + ether mixtures nine times, it had a m. p. of $82.5-83^\circ$. C, 78.14; H, 12.7. This acid is pure tetracosanic acid. It should be noted that *n*-tetracosanol is the predominant spinach alcohol.

SUMMARY.

1. In addition to spinasterol, spinach contains a second sterol, $C_{27}H_{46}O$, m. p., $145-148^\circ$, and $(\alpha)_{5461} = +7.65^\circ$. The corresponding acetate, m. p., $150-154^\circ$ and $(\alpha)_{5461} = +7.2^\circ$.

2. The predominant paraffin alcohol is *n*-tetracosanol. *N*-tetracosanic acid melts at $82.5-83^\circ$.

3. *N*-hexacosanol was identified and the constants reported for *n*-hexacosanic acid by Pollard, Chibnall and Piper and confirmed.

BIBLIOGRAPHY.

- (1) F. W. Heyl, E. C. Wise and J. H. Speer, *J. Biol. Chem.*, 82 (1929), 111.
- (2) E. Clenshaw and I. Smedley-Maclean, *Biochem. J.*, 23 (1929), 107.
- (3) D. L. Collison and I. Smedley-Maclean, *Ibid.*, 25 (1931), 606.
- (4) A. Pollard, A. C. Chibnall and S. H. Piper, *Ibid.*, 25 (1931), 2119.
- (5) M. C. Hart and F. W. Heyl, *J. Biol. Chem.*, 95 (1932), 311.
- (6) F. B. Power, F. Tutin and H. Rogerson, *J. Chem. Soc.*, 103 (1913), 1276.
- (7) A. Gascard, *Compt. Rend.*, 170 (1920), 1326.

RESEARCH LABORATORIES, THE UPJOHN COMPANY,
KALAMAZOO, MICHIGAN.

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