LXX. THE NATURE OF THE LIPOID MATTER EXTRACTED FROM GREEN LEAVES (SPINACH AND CABBAGE).

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The unsaponifiable fraction from the spinach leaf.

CLENSHAW and SMEDLEY-MACLEAN [1929] examined the unsaponifiable matter of the lipid matter of spinach leaves and isolated from it (1) carotene, (2) a saturated hydrocarbon melting at 68–68·5° which they identified as hentriacontane $C_{31}H_{64}$, and (3) a sterol which forms less than 5 % of the total unsaponifiable matter. The same authors also examined the leaves of green cabbage and showed that they contained a hydrocarbon, which they did not completely characterise, similar to that present in spinach. This was accompanied by a substance containing oxygen which did not appear to be represented in spinach and which was reserved for further investigation.

Shortly afterwards Heyl, Wise and Speer [1929] published the results of an examination of spinach fat in which very much larger quantities of material had been worked up than had been used by the previous observers. The conclusions of these authors differed so widely from those of Clenshaw and Smedley-MacLean that it seemed desirable to repeat the investigation of spinach fat and to endeavour to reconcile the discrepancies.

The hydrocarbon isolated by Heyl, Wise and Speer melted at 67·5–68·5° and contained C, 84·8 %, H, 15 %: to this they ascribed the formula $C_{20}H_{42}$, apparently on the ground of a single molecular weight determination by Rast's method. Since the analyses and melting-points of the hydrocarbons isolated in the two investigations were completely in agreement and corresponded with those of the hydrocarbon $C_{31}H_{64}$, it appeared necessary to carry out a number of determinations of the molecular weight of the hydrocarbon by Rast's method [1922] to see how far they agreed and also to confirm the composition of the hydrocarbon by submitting it to X-ray examination.

In our hands the values obtained for the molecular weight of the hydrocarbon were 409, 432 and 424, while another observer found 405 and 420 for the same material. We can offer no explanation of the value of 285, the single observation recorded by Heyl, Wise and Speer. Our experience indicates that while Rast's method is exceedingly useful in fixing the approximate molecular weight of a hydrocarbon, it does not afford a basis for a decision between

adjacent members of a homologous series when carried out under the ordinary laboratory conditions of determining melting-points.

We are indebted to Dr Piper for carrying out the X-ray examination. He reports that the hydrocarbon gave a very good photograph spacing $41.3 \text{ \AA}$ units to $\frac{1}{2} \%$. It gave a large number of orders—about ten measurable—and was probably a highly pure specimen of the hydrocarbon containing 31 carbon atoms.

There is no doubt therefore that Clenshaw and Smedley-MacLean were correct in assigning the formula $C_{31}H_{64}$ to the hydrocarbon present in the unsaponifiable matter of spinach. From the same material Heyl, Wise and Speer claim to have isolated two other substances; (1) an alcohol melting at $76-77^\circ$ to which they ascribed the formula $C_{22}H_{46}O$ as the result of analysis and of a single recorded observation by Rast's method, and (2) a substance melting at $87-88^\circ$ containing C, 77.7 %, H, 13.7 %, for the molecular weight of which a single determination by Rast's method gave 272. Presumably as the result of an error in calculation the formula $C_{24}H_{50}O_2$ corresponding with a molecular weight of 370 is ascribed to this substance. Having worked up considerably more material than in the earlier investigation of Clenshaw and Smedley-MacLean, we succeeded in isolating a substance containing oxygen, which agreed in melting-point and in composition with the alcohol described by Heyl, Wise and Speer. The complete separation of all traces of hydrocarbon from the spinach alcohol is attended with some difficulty. We found that the alcohol formed an acetate melting at 61.5° and a urethane melting at $64-65^\circ$ and closely resembled *n*-ceryl alcohol. A specimen of the latter was prepared from Chinese wax and the addition of this alcohol, its acetate and urethane respectively to the similar compounds derived from the spinach alcohol caused no depression in melting-point. The alcohol described by Heyl, Wise and Speer as $C_{22}H_{46}O$ is therefore identical with ceryl alcohol.

Alcohol	C %	H %	Mean mol. wt. (Rast)	M.P.
Ceryl alcohol ($C_{26}H_{54}O$)	81.67	14.13	382	$78-79^\circ$
Alcohol from Spinach:				
(a) Heyl, Wise and Speer	80.9	14.2	272	$76-77^\circ$
(b) Collison and Smedley-MacLean	81.32	13.74	353	$77-78^\circ$
			365	
			421	

In our investigation the total amount of dry spinach leaves worked up was 6.1 kg. whereas the American investigators started with 68 kg. This probably explains why we did not succeed in obtaining any of the oxygen-containing substance melting at $87-88^\circ$, 0.2 g. of which was isolated by Heyl, Wise and Speer.

The sterol from spinach was of particular interest. Unfortunately the amount isolated was not sufficient for an extended investigation. Our analyses agreed with those of the above observers and we confirm the formula $C_{27}H_{46}O$. It is dextro-rotatory, melts at 164° , gives a Salkowski reaction with the reddish

colour in the sulphuric layer, and is highly unsaturated giving an iodine value by Hubl's method of 245. It closely resembles in its properties the much lower-melting zymosterol with which it is isomeric.

The fatty acids of the spinach leaf.

These were examined and palmitic and cerotic acids identified, confirming the result of Speer, Wise and Hart [1929]. From 3600 g. dried spinach leaves, 69.12 g. of material soluble in light petroleum were extracted. On saponifying this with a cold alcoholic solution (6 %) of sodium ethoxide, 25.67 g. of unsaponifiable matter were obtained and after acidification and further purification by boiling with charcoal in solution in light petroleum, 10.5 g. of fatty acids were isolated. The acid fraction was separated by means of the solubilities of its lead salts in ether into a liquid and a solid fraction. The latter was separated into two parts which showed the following properties:

	M.P.	Mol. weight (by titration)
Fraction I	74.5–75.5°	406
(Several times recrystallised)	78–78.5°	—
Cerotic acid	78–79°	410
Mixed M.P.	78–79°	—
Fraction II	50°	266
(Several times recrystallised)	59–61°	—
Palmitic acid	62°	256

The molecular weight of the liquid portion of acids was found by titration to be 259 and its iodine value 205.8; on bromination of this portion, oleic and linolenic acids were identified. A doubly unsaturated acid was also present, but the solubility of its tetrabromide in light petroleum was greater than that expected for the bromide of linoleic acid. It is possible therefore that the doubly unsaturated acid present is either an isomeride of linoleic acid or else a lower homologue. The quantity of material at our disposal did not permit us to investigate this further.

The unsaponifiable fraction from the cabbage leaf.

The amount of lipid material extracted by light petroleum from green cabbage leaves which had previously been dried in air at 37° was similar to that which we had obtained from spinach leaves, namely about 2 %, of the weight of dried leaves. Approximately one half of this consists of unsaponifiable matter. Chibnall and Channon [1927, 1] carried out an investigation on the lipid matter of cabbage, prepared in a somewhat different manner. Their method was to mince the cabbage leaves, to add an equal volume of water to the minced leaves and to express the juice, which was then coagulated at 70°. The coagulum was separated by filtration and pressed so that the residue contained about 40 % of water. The residue when treated with ether gave an extract which was dark green in colour and contained chlorophyll and its decomposition products, whereas the extract which we obtained by the

direct treatment of the leaves (dried at 37°) with light petroleum was light yellow and almost free from chlorophyll.

The lipid matter from the petroleum extract was saponified in the cold by means of 6 % alcoholic sodium ethoxide and gave about half its own weight of unsaponifiable matter. The iodine value of the unsaponifiable matter obtained from the green leaves of large summer cabbage was 100 compared with a value of 57.5 for that obtained from the pale yellow heart leaves of the same cabbage. The iodine value of the unsaponifiable matter prepared from a green unheaded winter cabbage was 58.5. The difference in iodine value is probably explained by differences in carotene content [Collison, Hume, Smedley-MacLean and Smith, 1929] for the carotene content of white leaves is much less than that of green leaves and the winter cabbage also proved less effective as starting material for the preparation of carotene than the large-headed summer cabbage. The iodine value of carotene determined by the same method as that used for the unsaponifiable matter of cabbage was 330 and the proportion of carotene present in the unsaponifiable matter must therefore greatly influence the iodine value of the unsaponifiable material.

The unsaponifiable material was treated with hot alcohol: most of the carotene remained in the sticky insoluble residue and from this a specimen melting at 178° was prepared. In the hot alcoholic solution there remained the hydrocarbon and oxygen-containing compound previously noticed by Clenshaw and Smedley-MacLean. A full description of these substances was published by Channon and Chibnall [1929] shortly afterwards and they were identified as *n*-nonacosane, $C_{29}H_{60}$, and *n*-dimyristyl ketone respectively. We found that the best method of separating these substances is by washing the mixture with small quantities of cold light petroleum, in which the hydrocarbon is more readily soluble. When the mixture containing a large proportion of the ketone was converted to the oxime by the method described by Channon and Chibnall and the substance obtained after oximation dissolved in acetic acid, the precipitate which separated when the solution was cooled melted at 55°–60°. On the addition of alcohol to the filtrate, short needles separated melting at 57.5°. When these were mixed with palmitone oxime (m.p. 58–59°) the melting-point of the mixture was depressed by 5°. The oxime of the dimyristyl ketone described by Channon and Chibnall melted however at 50–51°. In another similar preparation of the oxime starting with a different batch of material, the oxime which separated melted at 52–53°. Both specimens, melting respectively at 52–53° and at 57.5°, gave the same results on analysis and from both on treatment with acid the same ketone melting at 80° was recovered. We are now investigating the reason for this difference in melting-point, which we cannot at present explain.

The fatty acids of the cabbage leaf.

From 2800 g. of dry cabbage leaves, 14.3 g. of fatty acids insoluble in water were separated and these were divided by the usual lead salt method into 7.21 g. of liquid and 1.6 g. of solid acids.

The solid acid fraction was separated by repeated crystallisation into two parts melting respectively at 62–64° and 80–80.5°. So little material was available that the definite identification of these fractions presented considerable difficulties. The lower melting fraction appeared to consist chiefly of palmitic acid. The molecular weight determined by titration of the fraction of m.p. 80° was 357, a value intermediate between those required for behenic acid ($C_{22}H_{44}O_2$, m.p. variously given as 77–78°, 80–82°, 84°) and lignoceric acid ($C_{24}H_{48}O_2$, m.p. 80.5°). An ethyl ester was prepared from the small amount of acid available and melted at 51–52°. The ethyl ester of behenic acid melts at 56–57°.

Chibnall and Channon [1927, 2] give the following figures for the identification of the fractions from the 1.45 g. of solid acids isolated by them from cabbage:

		C %	H %	M.W.	M.P.
Fraction (1)	0.38	75.23	12.60	271	54–55°
(2)	0.65	75.18	12.43	262	54–55°
(3)	0.34			266	—

They point out that these figures would be given by a mixture of 30 % of stearic and 70 % of palmitic acid and hence they assume that these acids are present. A mixture of 90 % palmitic acid with 10 % behenic acids would give the following figures:

C %	H %	M.W.
75.19	12.61	264

From the evidence now brought forward it is clear that the evidence for the presence of stearic acid in the cabbage leaf is very doubtful, since in this plant the palmitic acid present is associated with a small proportion of a higher saturated acid, probably behenic.

In view of the recent identification of the liquid acids as oleic, linoleic or its homologue, and linolenic, we did not further investigate the liquid fraction of the acids.

General remarks on the lipoid material extracted from green leaves.

From the investigation of the leaves of cabbage and spinach and from the evidence available, chiefly in the work of Power, Tutin and their collaborators, it seems probable that the higher members of the normal paraffin series and their derivatives must play an important part in the metabolism of the plant leaf.

Speaking generally the bulk of the acids of the leaf seem to be the unsaturated acids. Linolenic, linoleic or an isomeride, oleic and an unsaturated acid containing 16 carbon atoms have been recorded. The proportion of solid acids is small and of these palmitic acid seems to be an almost invariable constituent and in a large proportion of instances has been isolated in the pure state. Smaller amounts of one or other of the higher saturated fatty acids appear commonly to be present. Stearic acid is frequently noted though the evidence for its presence often consists of the analysis of a fraction of fatty acids of lower melting-point and slightly higher carbon content than palmitic acid. This mixture is sometimes obtained by recrystallisation and sometimes by saponification of a fraction obtained by distillation of the methyl esters, but in many cases the evidence adduced for the presence of stearic acid is unconvincing. From the evidence in the literature cerotic appears to occur most commonly of the higher fatty acids and several instances are recorded of the occurrence of arachidic, behenic, and melissic acids.

The higher fatty alcohols also occur; ceryl alcohol has been found in spinach leaves in the present investigation, in hops [Power, Tutin and Rogerson, 1913], in *Prunus serotina* [Power and Moore, 1909] and in other plants.

The occurrence of these higher fatty alcohols and acids is of interest in connection with the presence of the higher paraffin hydrocarbons in leaves. Thorpe and Holmes [1901] isolated from tobacco leaves two hydrocarbons melting respectively at 59.3–59.8° and 67.8–68.5°, and identified them as the normal paraffin hydrocarbons containing 27 and 31 carbon atoms. Since that time, the analyses of a large number of leaves, stems and flowers have been made chiefly by Power, Tutin and their collaborators. In a somewhat cursory survey of the literature we have noted the isolation of these hydrocarbons from 39 different plants. From 21 of these hentriacontane ($C_{31}H_{64}$), melting at 68° or in its near neighbourhood, has been separated. This hydrocarbon is the one most often recorded and is presumably derived from palmitic acid, by conversion of the latter to palmitone and by the subsequent reduction of the latter. We can however in the papers we have examined find no record of the presence of palmitone in the same material and we have been unable to find any evidence of the presence of this ketone in the spinach leaf. The presence of pentatriacontane $C_{35}H_{72}$ which bears a similar relation to stearic acid to that existing between hentriacontane and palmitic acid is recorded nine times: heptacosane $C_{27}H_{54}$ presumably derived from myristic acid appears five times. Nonacosane $C_{29}H_{60}$, M.P. 63–64°, was isolated by Pickles [1911] from the leaves and stems of bog myrtle and recently by Channon and Chibnall from cabbage. There also occurs nine times the description of triacontane $C_{30}H_{62}$, a hydrocarbon which alone of those described contains an even number of carbon atoms and which could not therefore be derived by the method postulated from any one single fatty acid. The evidence for the existence of this seems weaker than for that of any of the other hydrocarbons described and it appears not improbable that the so-called triacontane should really be

regarded as nonacosane, since the evidence rests chiefly on the determination of the melting point and this in all cases approximates closely to that of the nonacosane.

	M.P.	Source	
Nonacosane	63-64°	Bog myrtle	Pickles [1911]
	(uncorr. 62.7-62.8°)	Cabbage	Channon and Chibnall [1929]
Triacontane	64-65°	<i>Anthemis nobilis</i>	Power and Browning
		flowers	[1914, 2]
	60-61°	Cotton-root bark	" " [1914, 1]
		<i>Euphorbia pilulifera</i>	" " [1913]
	63-65°	<i>Matricaria chamomilla</i>	" " [1914, 3]
		flowers	
	65.2°	<i>Eriodictyon</i> leaves	Power and Tutin [1907]
	65.5°	<i>Cluytia similis</i>	Tutin and Clewer [1912]
	65.5°	<i>Solanum angustifolium</i>	" " [1914]
		leaves and flowers	

Since Levene, West and van Scheer [1915] find that the melting-point of synthetic triacontane is 69-70°, the examination of the naturally occurring hydrocarbon which has been identified as triacontane needs revision; there are certainly grounds for believing that it may be identical with nonacosane.

If triacontane may be eliminated, the existence of hydrocarbons containing only odd numbers of carbon atoms agrees with their derivation from the fatty acids containing respectively 14, 15, 16 and 18 carbon atoms. The difficulty of obtaining the solid saturated acids from leaves in sufficient quantity to make a complete identification renders it difficult to say whether the acid with 15 carbon atoms does occur in the plant leaf. Acids with odd numbers of carbon atoms are described, as for instance, that with 21 carbon atoms which is several times recorded [*e.g.* Tutin and Clewer, 1912] and it may well be that α -oxidation of palmitic acid takes place in the plant leaf. The occurrence of dimyristyl ketone in the cabbage leaf together with the hydrocarbon derived from it by reduction is of great interest and suggests that the fatty acid containing 15 carbon atoms must occur at some stage in the metabolism of the leaf.

SUMMARY.

1. The unsaponifiable matter of the spinach leaf contains in addition to carotene and a sterol, $C_{27}H_{46}O$, the hydrocarbon hentriacontane, $C_{31}H_{64}$, the identification of which has been confirmed by X-ray examination. Ceryl alcohol $C_{26}H_{54}O$ is also present. Palmitic, cerotic, oleic, linolenic acids and an isomeride of linoleic acid have been isolated.

2. The identification of nonacosane and *n*-dimyristyl ketone in the unsaponifiable matter of the cabbage leaf [Channon and Chibnall, 1929] is confirmed. The saturated fatty acids are palmitic and a higher fatty acid, probably behenic.

3. The question of the occurrence of the higher paraffin hydrocarbons in the leaf and their probable derivation is discussed.

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carry out this work and to express our thanks to Dr Piper for his kindness in carrying out the X-ray examination of the hydrocarbon.

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