LXIX. THE ETHER-SOLUBLE SUBSTANCES OF CABBAGE LEAF CYTOPLASM.

III. THE FATTY ACIDS.

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A METHOD of preparing the ether extract of leaf cell cytoplasm in bulk from green unheaded cabbage (*Brassica oleracea*) has been described in a previous paper [Chibnall and Channon, 1927, 1]. A table showing some of the fat constants of the material was given, and it was pointed out that the addition of 4 volumes of acetone to the ether extract results in the precipitation of about 40 % of the material. The chemical nature of certain of the constituents of this precipitate has already been discussed [Chibnall and Channon, 1927, 2] and further investigations of the remaining substances present in it are in progress. The fraction of the extract which is not precipitated from ether by acetone—some 60 % of the whole—contains the fatty acids which are not in complex combination in phosphorus-containing substances. It is with these fatty acids that this communication deals.

On evaporation of the ether-acetone extract *in vacuo*, there is obtained a hard, waxy mass which is almost black in colour on account of the presence in it of chlorophyll. It would obviously have been desirable to remove the pigment before proceeding further, but as this was impossible without the loss or chemical alteration of some of the other substances present, it was necessary to use this pigmented material without further treatment.

Preparation of the fatty acids and the unsaponifiable matter.

After removal of the ether and acetone *in vacuo*, the material was saponified with excess of strong alcoholic potash for 1 hour, and the alcoholic solution poured into water. From this aqueous solution the unsaponifiable matter was removed by exhaustive extraction with ether and the ether extract washed with water. The water washings were united to the main bulk of the aqueous soap solution. On evaporation of the ether solution, there remained a hard orange brown mass which was re-saponified with 2 N sodium ethylate, and then extracted as before. The aqueous solution and the washings from the

ether extract were united to the main bulk of the soap solution obtained during the initial saponification. On removal of the ether, there was obtained the unsaponifiable fraction, the nature of which will be discussed in a subsequent communication.

The aqueous solution, after the removal of the unsaponifiable matter, will contain not only the soaps produced by the saponification of all the esters present in the free fat, but also the alkali salts of the acids produced by the degradation of the chlorophyll originally present. On acidification of the soap solution therefore, there will be precipitated not only the fatty acids but also the chlorophyll derivatives, which Willstätter's researches [Willstätter and Stoll, 1913] indicate will be phytochlorin *e*, $C_{32}H_{32}ON_4(COOH)_2$ and phytorhodin *g*, $C_{32}H_{30}O_2N_4(COOH)_2$. Ether extraction of the soap solution removes the fatty acids and these "chlorophyll" acids. After the usual treatment of the ether extract, there remains behind a black fatty mass which can be fractionated into products soluble and insoluble in light petroleum.

The former are the fatty acids whilst the latter are the chlorophyll derivatives with, possibly, hydroxylated acids. The following table illustrates the general nature of the unsaponifiable matter and the fatty acids, obtained by the foregoing methods from the ether-acetone filtrate.

Table I.

Batch	Autumn sown (picked April and May)				Spring sown (picked August)	
	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>K</i>	<i>M</i>
% of total ether extract not precipitated by acetone	59.5	58.0	59.0	58.5	56.0	60.1
Iodine value	141	147	152	143	154	—
Saponification value	151	158	152	—	149	—
% of unsaponifiable matter	16.9	16.5	17.0	17.1	24.1	25.0
% sterol	3.63	2.65	3.70	3.61	3.80	3.70
Iodine value	113	117	119	122	105	97
% fatty acids	23.2	23.1	23.0	23.6	15.6	16.3
Iodine value	173.5	203.5	201	197	206	—
Neutralisation value	209	206	198	—	197	—

From this table, it will be seen that the general nature of the ether extract in all the batches is very similar save that there appears to be a definite difference in the amounts of the fatty acids and of the unsaponifiable matter in the material obtained during the spring and summer.

Fatty acids soluble in light petroleum.

By evaporation of the solvent, the fatty acids remain as a pale yellow, almost liquid mass, free from chlorophyll degradation products.

Saturated acids. 7.8 g. of batch *H* and 5.7 g. of batch *E*, 13.5 g. of fatty acids in all, having an iodine value of 205, were treated by the method of Levene and Rolf [1922]. By fractionation of the barium soaps in benzene containing 5 % of 95 % alcohol, there were obtained 4.63 g. of more saturated

acids having an iodine value of 163, 7·87 g. of less saturated acids having an iodine value of 218. The result of this treatment of the barium soaps shows that the separation has not been very satisfactory. This is not surprising when it is known that by far the greater bulk of the fatty acids consists of acids of 18 carbon atoms containing 2 and 3 double bonds; the precipitate of the barium soaps of the saturated acids and oleic acid contains, therefore, a considerable amount of the barium soaps of these unsaturated acids. The 4·63 g. of more saturated acids were further fractionated by conversion into the lead soaps and treatment of these soaps with ether. The soaps insoluble in ether yielded 1·45 g. of solid acid, which was separated into three fractions by crystallisation from 95 % alcohol. These fractions weighed 0·38 g., 0·65 g. and 0·34 g. respectively, and they gave the following analyses:

Fraction (1)	0·0854 g. substance	0·2356 g. CO ₂ and 0·0969 g. H ₂ O
	0·3772 g. "	required 1·95 cc. $\frac{1}{2}$ N KOH for neutralisation
(2)	0·0822 g. "	0·2266 g. CO ₂ and 0·0914 g. H ₂ O
	0·4802 g. "	required 2·56 cc. $\frac{1}{2}$ N KOH for neutralisation
(3)	0·3402 g. "	1·79 cc. $\frac{1}{2}$ N KOH "
Calculated	C ₁₆ H ₃₂ O ₂ : C, 74·92; H, 12·58.	M.W. 256; M.P. 63·4°
	C ₁₈ H ₃₆ O ₂ : C, 75·93; H, 12·78.	M.W. 284; M.P. 70–71°
Found. Fraction (1)	C, 75·23; H, 12·60.	M.W. 271; M.P. 54–55°
(2)	C, 75·18; H, 12·43.	M.W. 263; M.P. 54–55°
(3)		M.W. 266

The amount of material available was obviously too small for the definite identification of the saturated acids present, but the results would suggest that they consist of about 70 % palmitic together with 30 % of stearic acid, the melting-point of which mixture is given as 56° [Lewkowitch, 1921]. It will be seen that 13·5 g. of the acids have given 1·45 g. of saturated acid.

Unsaturated acids. The liquid acids generated from the lead soaps soluble in ether were again fractionated by the barium soap-benzene method. The insoluble barium soaps yielded only 0·225 g. of fatty acid having an iodine value of 127. As oleic acid has an iodine value of only 90, it is clear that only a part of this 0·225 g. can be oleic acid; indeed, having regard to the fact that the bulk of the fatty acids are highly unsaturated, it might well be that this 0·225 g. consists not of oleic acid but of saturated acid contaminated by those unsaturated acids.

The 9·2 g. of less saturated acids obtained from the two fractions of the barium soaps soluble in benzene-ether were brominated in anhydrous ether at 0° and were then separated into three fractions: (1) 4·95 g., insoluble in ether, (2) 10·0 g., insoluble in light petroleum, (3) 3·22 g., soluble in light petroleum. These fractions gave the following analyses:

Fraction (1)	0·0879 g. substance	0·0866 g. CO ₂ and 0·0301 g. H ₂ O
	0·1244 g. "	required (Stepanoff) 10·1 cc. N/10 thiocyanate
(2)	0·4133 g. "	" " 28·1 cc. " "
(3)	0·1458 g. "	" " 8·2 cc. " "
Calculated	C ₁₈ H ₃₀ O ₂ Br ₆ : C, 28·49; H, 3·99; Br, 63·26;	M.P. 180–181°
	C ₁₈ H ₂₆ O ₂ Br ₄ : C, 28·49; H, 3·99; Br, 53·28;	M.P. 113–114°
	C ₁₈ H ₃₄ OBr ₂ : C, 28·49; H, 3·99; Br, 36·2	
Found. Fraction (1)	C, 28·12; H, 3·88; Br, 63·02;	M.P. 180°
(2)		Br, 54·30
(3)		Br, 44·9

The mono-, di- and tri-glycerides of the C_{18} saturated acids give the ratios 3.1/1, 6.2/1 and 9.3/1. A discussion of the glycerides present in the original ether extract will be held over until the analysis of the fraction which may contain hydroxy-acids is complete.

SUMMARY.

The fatty acids of the cytoplasm of the cabbage leaf have been studied; the greater part consists of the unsaturated acids linolenic and linolic acids; palmitic and stearic acids constitute the saturated acids; the presence of oleic acid has not been proved. The ratio of fatty acids to glycerol is 4.6 : 1.

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