available prior to partition chromatography. If this were the case, the only outstanding difference between the composition of leaf proteins and the algal protein reported here is in the very low cystine content of the latter. The composition of the yeast protein also shows many similarities with that of *Chlorella*, the values for the monoamino-monocarboxylic acids again being much higher than those reported for the leaf proteins.

SUMMARY

1. A representative sample of the proteins of the alga *Chlorella vulgaris* was prepared.

2. The amino-acid composition of this protein fraction was investigated by the technique of paper-partition chromatography after an acid hydrolysis during which no humin formation occurred.

3. The analysis accounted for 101·3% of the total protein nitrogen.

4. The amino-acid composition of this algal protein fraction is compared with figures available for the composition of the proteins obtained from the leaves of the higher plants and of the proteins found in a sample of a brewer's yeast.

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The Branched-chain Fatty Acids of Butterfat

2. THE ISOLATION OF A MULTI-BRANCHED C_{20} SATURATED FATTY ACID FRACTION

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In Part 1 of this series (Hansen & Shorland, 1951) the occurrence of small amounts of branched-chain acids in butterfat was indicated and the isolation of two C_{17}-methyl branched-chain acids, isomeric with normal heptadecanoic acid, was reported.

Further investigation of the acetone-soluble components separated at low temperature from hydrogenated methyl esters of the C_{18} acids of butterfat, has revealed the presence of a multi-branched C_{20} acid fraction.

EXPERIMENTAL

As reported earlier (Hansen & Shorland, 1951) a concentrate (4·4 kg.) of methyl esters of C_{18} acids prepared from butterfat was crystallized from acetone at −30°. The soluble portion after hydrogenation was again crystallized from acetone at −30° yielding 87·6 g. of acetone-soluble material with iodine value 8·3. This latter fraction was distilled *in vacuo* in an 8 ft. Vigreux column yielding eight fractions, EL1 to EL8, together with a viscous residue ELR (compare Table 1, Hansen & Shorland, 1951). From these eight fractions were derived the C_{17} branched-chain acids previously reported, together with all subsequent fractions referred to in this paper. By repetition of the processes of selective bulking of those fractions with similar molecular weight, followed by fractionation, a series of fractions was obtained which could not be further resolved by the columns at our disposal and which were combined to constitute E4 (9·6 g. methyl esters, mean saponification equivalent 303·4).

The methyl esters of E4 were crystallized from 20 vol. of methanol at −37° yielding: (*a*) E4L 5·56 g., soluble

Table 1. *Fractionation of methyl esters* (E7L, 1·5 *g.*)

Fraction	Weight (g.)	B.p. (°) (approx. 0·1 mm.)	Saponification equivalent	Refractive index	Iodine value	
E7L1	0·06	120–125	—	—	—	—
E7L2	0·30	125–128	328·2	$n_D^{20°}$ 1·4425	—	Liquid
E7L3	0·95	128	329·9	$n_D^{20°}$ 1·4426	1·6	Liquid at −27°
E7L4	0·19	128	330·5	$n_D^{20°}$ 1·4431	—	Liquid

Table 2. *Chemical and physical properties of fatty acid fraction* E9

Fraction	Wt. (g.)	Sap. equiv.	Iodine* value (Wijs)	C-methyl†	Combustion analysis	M.p. (°)	Refractive index	Optical rotation
E9 (acid)	1·05	312·7	0·4	13·09%	C 77·4% H 12·9%	Below −70	$n_D^{20°}$ 1·4500	Nil

* $E_{1\text{ cm.}}^{1\%}$ 234 mμ. value was 5·9, indicating the presence of only trace amounts of conjugated unsaturation.
† Analyses by Weiler & Strauss, Oxford, England.

Table 3. *Chemical and physical properties of fatty acid fraction* Eq1bL

Fraction	Wt. (g.)	Sap. equiv.	Iodine value	C-Methyl*	Combustion analysis*	Refractive index	M.p. (°)	Optical rotation
Eq1bL (acid)	0·19	314·5	Nil	13·4%	C 77·2% H 13·1%	$n_D^{20°}$ 1·4513	Below −70	Nil

* Analyses by Weiler & Strauss.

fraction, liquid at room temperature, saponification equivalent 310·5. (*b*) E4S 4·02 g., insoluble fraction, also liquid at room temperature, saponification equivalent 294·7.

Chromatographic adsorption on activated alumina of E4L dissolved in light petroleum (b.p. 50–60°), served to remove traces of impurities and effected separation into nineteen fractions. Two of these fractions E4Lb 0·89 g. $n_D^{20°}$ 1·4424, and E4Lc 1·28 g. $n_D^{20°}$ 1·4425, which had been eluted with light petroleum (b.p. 50–60°) were combined together and denoted E7 (saponification equivalent 324·3).

Crystallization from 20 vol. of methanol at −60° resolved E7 into two fractions: (*a*) E7L 1·88 g., soluble fraction, liquid at room temperature, m.p. below −35°, and (*b*) E7S 0·04 g., insoluble fraction, solid at room temperature.

E7L (saponification equivalent 325·1, iodine value (Wijs) 1·1) when dissolved in 20 vol. of light petroleum (b.p. 50–60°) gave no precipitate at −70°. Fractionation of 1·5 g. E7L methyl esters in a microcolumn yielded four fractions as shown in Table 1.

Fraction E7L3 was crystallized from 20 vol. acetone at −60° giving (*a*) E7L3L 0·77 g., soluble fraction, and (*b*) E7L3S 0·01 g., insoluble fraction, E7L3L was completely soluble in 20 vol. light petroleum at −70°.

E7L3L and E7L2 were bulked together as acids and denoted E9, the chemical and physical characteristics of which are recorded in Table 2.

A second fraction Eq1bL, possessing properties closely agreeing with those of E9 has been derived by similar but independent processes from the original fractions already described (compare Table 1, Hansen & Shorland, 1951). Characteristics of fraction Eq1bL are recorded in Table 3.

DISCUSSION

The two fractions isolated (E9 and Eq1bL) display almost identical chemical and physical properties. Analytical data are in agreement with the empirical formula $C_{20}H_{40}O_2$, the theoretical values being C, 76·9; H, 12·9%; saponification equivalent 312·5.

The recorded C-methyl values for E9 and Eq1bL are equivalent to 2·78 and 2·81 mol. of acetic acid, respectively. These figures when interpreted according to the evidence of Ginger (1944), indicate the presence of at least three and possibly four methyl or homologous groups.

The structure of the multi-branched C_{20} acid fraction reported in this paper has not been established. Its extraordinarily low melting point (below −70°) contrasts it with Velick's (1944) phytomonic acid, $C_{20}H_{40}O_2$, which has a single methyl side chain and a melting point of 24° (more than 50° lower than that of the corresponding straight-chain acid eicosanoic, melting point 75·3°). From the evidence presented by Cason & Winans (1950) on the methyl substituted isomers of octadecanoic acid, it may be inferred that a low melting point will indicate branching near the centre of the straight chain. Multiple branching can be expected to be accompanied by further lowering of the melting point.

Characteristic of the fraction isolated is its extreme solubility in different solvents, suggesting that both the number of side chains and their positions profoundly influence the solubility of saturated acids.

The very low solidifying point of this C_{20} saturated branched-chain acid has precluded melting-point determinations, and in the absence of these criteria of homogeneity, freedom from isomers or closely related substances cannot be assured. When larger quantities of the substance become available, however, derivatives will be prepared and further resolution attempted.

SUMMARY

A C_{20} saturated acid fraction containing at least three and possibly four methyl groups has been isolated from butterfat.

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The Oxidation of Amines by Extracts of Pea Seedlings

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While a number of enzymes catalysing reactions which lead *in vitro* to hydrogen peroxide formation have been shown to be present in animal tissues little is known about the distribution of such enzymes in higher plants. These enzymes may be of importance not only because of the oxidations they catalyse, but also because the hydrogen peroxide formed can be used by peroxidase (Thurlow, 1925) or catalase (Keilin & Hartree, 1936, 1945) for coupled oxidations. In this connexion Kenten & Mann (1949, 1950) showed that peroxidase systems can oxidize manganese and suggested that hydrogen peroxide produced by flavoprotein enzymes could be used *in vivo* for this oxidation. In the present work colorimetric evidence has been obtained suggesting the presence of enzyme systems producing hydrogen peroxide in extracts of many plants. In an investigation of such enzyme systems in extracts of pea seedlings it has been found that not only diamines (Werle & Pechmann, 1949), but also certain monoamines are oxidized by these extracts with formation of hydrogen peroxide. The oxidation of the monoamines is apparently not due to the plant monoamine oxidase described by Werle & Roewer (1950).

MATERIAL AND METHODS

Plant material. The majority of the plants examined were glasshouse grown in a John Innes potting compost. The rest of the plants were from gardens in the neighbourhood of Rothamsted. The peas and beans were soaked for 24 hr. in water prior to sowing. In some experiments with peas when seedlings only a few days old were required the peas were germinated in moist washed sand. Pea seedlings were also grown aseptically in boiling tubes on a mineral salts agar medium. Before sowing on this medium the seed coats were sterilized by treatment first with ethanol and then with 0·2% $HgCl_2$ washed off with several changes of sterile water (Thornton, 1930).

Enzyme preparations. Water extracts of the plant material and a dry preparation obtained by precipitation of such extracts with $(NH_4)_2SO_4$ and acetone were used.

(*a*) *Plant extracts.* The plant material was washed free from soil and ground in a mortar with sand and water, squeezed by hand through madapollam and finally made up to approximately double the wet weight.

(*b*) *Dry prepration.* Pea seedlings (6–18 days old) were washed free from soil and any obviously diseased material removed. With the older seedlings it was frequently necessary to remove a number of the cotyledons on this account. About 100 g. of seedlings were extracted as above; the extract was then fractionally precipitated with $(NH_4)_2SO_4$ and the precipitate obtained with 30–66% saturation was dialysed overnight against 2 l. of distilled water. The dialysed suspension was centrifuged and the supernatant cooled to 0° and treated with 2 vol. of acetone cooled to −10°. The precipitate was centrifuged off and dried *in vacuo*. The yield was 400–600 mg. The activity of the various fractions was estimated manometrically by measuring the increase in O_2 uptake produced by 0·01 M-β-phenylethylamine in 0·067 M-orthophosphate at pH 8·5. The supernatant obtained after dialysis of the $(NH_4)_2SO_4$ precipitate contained 60–70%, and the acetone precipitate 30–40%, of the activity of the original plant extract. The dry preparation was stored *in vacuo* at room temperature, and under these conditions about 30% of the activity towards amines was lost in a fortnight.