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

V. THE ISOLATION OF *n*-NONACOSANE AND DI-*n*-TETRADECYL KETONE.

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IN two of the earlier papers of this series we have described the phosphatides present in the fraction of the ether extract obtained by adding excess of acetone to the ethereal solution. This so-called phosphatide fraction contains in the present case two substances which do not contain phosphorus, and in this paper we describe our investigations into the chemical nature of these compounds which appear in the phosphatide fraction by reason of their insolubility in ether-acetone.

These substances, which may represent nearly one-half of the fraction or one-fifth of the total ether extract, can be readily separated from the true phosphatide material by extraction with boiling acetone, in which they are very readily soluble. On cooling, they separate at once in crystalline form, and it is shown that the main constituents are the paraffin *n*-nonacosane $C_{29}H_{60}$ and the closely allied di-*n*-tetradecyl ketone $C_{14}H_{28}.CO.C_{14}H_{28}$. The presence of such large amounts of nonacosane is surprising, for although higher paraffins are very widely distributed in the plant kingdom, they have been generally isolated in extremely small amounts. Nonacosane has been repeatedly isolated from various plant organs and identified by its melting point, but as Piper and his co-workers [1925] have shown that determinations of melting point and molecular weight are insufficient to define absolutely the number of carbon atoms in the constituent paraffins of paraffin wax, it is probable that the identification of most of these naturally occurring paraffins remains uncertain. At present the number of carbon atoms in such a substance can be determined only by X-ray analysis, and we have to thank Mr S. H. Piper for the final identification of the cabbage paraffin as nonacosane.

The separation of the ketone from the paraffin and its absolute identification as di-*n*-tetradecyl ketone have given great trouble on account of the very similar physical properties of the two substances. It was finally accomplished by taking advantage of the greater solubility of the ketoxime in a mixture of equal parts of light petroleum and acetone, a solvent in which the paraffin is practically insoluble. The regenerated ketone (m.p. 80.5–81°)

was similar in all its properties to synthetic palmitone (m.p. 82–82.5°), but a mixed melting point (76°) showed that the ketones were not identical. Resort was again made to X-ray analysis, and Mr Piper was able to show firstly that the ketone, like the hydrocarbon, contained 29 carbon atoms, and secondly that the carbonyl group was in the centre of the chain. The ketone chain however is so long that he could not be certain whether the ketone was the symmetrical di-*n*-tetradecyl ketone $\text{CH}_3 \cdot (\text{CH}_2)_{13} \cdot \text{CO} \cdot (\text{CH}_2)_{13} \cdot \text{CH}_3$ or the unsymmetrical palmityl myristyl ketone $\text{CH}_3 \cdot (\text{CH}_2)_{14} \cdot \text{CO} \cdot (\text{CH}_2)_{12} \cdot \text{CH}_3$. It seemed not unreasonable to think that the hydrocarbon and the ketone were synthesised in the plant from fatty acids by a reaction in which two molecules of fatty acid condense to yield a ketone, which on reduction would be converted to a hydrocarbon. This may be expressed thus:

If the ketone were palmityl myristyl ketone, then the synthesis in the plant of nonacosane from one molecule of palmitic acid and one of myristic acid—both of which occur in plant products—seemed a likely supposition. On the other hand, if the ketone were the symmetrical di-*n*-tetradecyl ketone, it would be necessary to postulate a synthesis involving two molecules of pentadecylic acid, and it is well known that the naturally occurring higher fatty acids up to and including stearic acid contain an even number of carbon atoms. Although the higher paraffins have been isolated from a large number of plant sources, no evidence has been forthcoming as to their origin in the plant, and the lack of a reasonable hypothesis for their metabolism made it appear vital that the exact position of the carbonyl group should be established with certainty. Accordingly the alternative ketones suggested by Piper were synthesised and a comparison of the melting points left no doubt that the cabbage ketone was di-*n*-tetradecyl ketone. We have at present no evidence of the existence in natural products of pentadecylic acid. It is true that Thörner [1879] isolated the so-called lactic acid from fungi and that a ketone, lactarone $(\text{C}_{14}\text{H}_{29})_2 : \text{CO}$ (m.p. 81.5–82.5°), was made by Chuit [1889] from this acid. Bougault and Charaux [1912] have since shown, however, that this acid is impure stearic acid, so that the ketone which is quoted in Beilstein as di-*n*-tetradecyl ketone is really impure stearone. Hence the hypothesis that the ketone is formed from two molecules of pentadecylic acid needs modification. It seems reasonable however to surmise that the paraffin and ketone are concerned in the metabolism of fatty acid, and, although we are unable to express the mechanism by which these three types of compound are formed from each other, we are of the opinion that the finding of a paraffin and a ketone of the same number of carbon atoms in the cabbage may prove significant when further information is available.

A search of the literature shows that what we refer to in the experimental part of this paper as the crude hydrocarbon, *i.e.* the mixture of paraffin and

ketone, has been encountered by other workers. Sando [1923] examined the wax-like coating on the surface of apples. One of his crude fractions had the same melting point and gave the same combustion figures as those quoted later for the crude hydrocarbon from the cabbage; from it he isolated a paraffin (M.P. 63.5–64°) which he regarded as $C_{30}H_{62}$. We feel confident also that the ketone $C_{53}H_{106}O_2$ isolated by Hesse [1893] from cocoa leaves and the so-called myristone obtained by Jacobson [1911] from lucerne were likewise mixtures of paraffin and ketone.

EXPERIMENTAL.

220 kg. leaves from winter-sown cabbage picked in May (batch *R*) yielded 220 g. of ether-soluble substances. The fraction insoluble in ether-acetone was extracted with hot acetone and filtered. The investigation of the insoluble phosphatide material has already been discussed [Channon and Chibnall, 1927]. The 35 g. of crystalline material obtained by cooling the hot acetone extracts was greenish in colour and obviously contained some fatty and phosphatide substances. The material was therefore saponified with alcoholic potash, allowed to stand overnight and filtered, the residue being washed with cold alcohol. By recrystallisation from hot acetone there was obtained 30 g. of material which was distilled at 255°/2 mm. The distillate (27 g.) was similar in appearance to, and had the same solubility properties as, paraffin wax. Recrystallised from a large volume of hot acetone, it yielded 23.9 g. of fine silky needles which melted at 63°, although the melt did not clear until a temperature of 67° was reached. The material was only slightly soluble in cold methyl and ethyl alcohols or acetone, slightly more soluble in cold ether, benzene or chloroform, but extremely soluble in hot ether, acetone or benzene. The ethereal solution did not absorb bromine, and did not give the Liebermann-Burchard reaction for sterols. Analysis showed however that although the major part of the material must consist of one or more hydrocarbons, some other substance which contained oxygen was present (found: C, 84.35, 84.28 %; H, 14.38, 14.47 %). The nature of this oxygenated body long eluded us. At the outset, we were deceived by an acetyl value of 12 into devoting considerable time in an attempt to remove higher alcohols, and much material was unfortunately wasted before we obtained clear evidence that the major part of the oxygen was to be ascribed to a ketone.

Determination of melting point. As we have found this determination so helpful in the fractionation of the crude hydrocarbon, we think that a short description of the method employed, which was recommended to us by Prof. Francis, may be of use to other workers in judging the purity of fractions of this type, which so often are isolated from plant products in small amounts only. The temperature of the bath is rapidly raised to within two degrees of the melting point, and then allowed to rise very slowly. A pure paraffin should melt to a clear liquid within the limits of 0.3° and should crystallise

out again in long needles within a temperature fall of 0.5° below the lower of these limits. The processes of melting and setting within these limits are slow, and the temperature of the bath must be held steady for several minutes. The ketone should melt to a clear liquid within a range of 0.5° , and with a nucleus should set crystalline within a temperature fall of 1° . A mixture of the paraffin and ketone becomes a white molten mass at a temperature which may be in some cases only 0.5° , but in others as much as $12\text{--}15^{\circ}$ below the temperature at which the melt clears. According to our experience, it is only a mixture of the paraffin with the ketone which gives an indefinite melting point of this type; mixed ketones and probably mixed paraffins give sharp melting and setting points. We have no hesitation in saying that the ketone isolated by Jacobson [1911], which softened at 68° and melted at $74\text{--}77^{\circ}$, is a mixture, and that its identification as myristone (M.P. 76°) is unwarranted. We have encountered many fractions giving similar melting points and combustion figures during the present research.

Isolation of nonacosane, $C_{29}H_{60}$.

13 g. of the crystalline material mentioned above were submitted to fractional distillation at a pressure of 0.1 mm., and crystallisation from benzene-alcohol. Seven distillations were made in all, and there was finally obtained, from the lower boiling fractions, 1.5 g. of substance crystallising in very thin glistening plates which melted sharply at $62.7\text{--}62.8^{\circ}$ (uncorr.) and set without a nucleus at $62.6\text{--}62.3^{\circ}$.

Analysis. 0.1057 g. substance gave 0.3307 g. CO_2 , 0.1387 g. H_2O .

Found: C, 85.33 %; H, 14.58 %.

$C_{29}H_{60}$ requires: C, 85.20 %; H, 14.80 %.

Mr Piper reports on this substance as follows. "Taking λ , $K_a Fe = 1.932 \text{ \AA}$, main spacing of melted specimen = $38.82 \text{ \AA}$. The hydrocarbon contains 29 carbon atoms and the photograph indicates that it is pure." The substance is therefore *n*-nonacosane, $C_{29}H_{60}$.

Isolation of di-n-tetradecyl ketone, $CH_3.(CH_2)_{13}.CO.(CH_2)_{13}.CH_3$.

The higher boiling fractions after a long fractional crystallisation from benzene-alcohol were finally collected into six samples. Melting point determinations showed that each sample contained nonacosane mixed with one or more other substances, for all became molten around 63° , but the temperatures at which the melts cleared ranged from 63.5 to 75° . It was clear that the procedure adopted was not likely to yield any of these other substances, which analysis showed contained oxygen, in a state of purity. When evidence was finally obtained that one or more of the substances was a ketone, it was found possible to effect a separation by taking advantage of the greater solubility of the ketoxime in a mixture of light petroleum and acetone.

Each of the fractions mentioned above was treated in alcoholic solution with hydroxylamine, the excess of the latter removed, and the product

dissolved in a small volume of light petroleum. An equal volume of acetone was then added and the resulting precipitate filtered off. By repeating this operation on the precipitate, and also on the filtrate, there was finally obtained from each fraction a somewhat crude hydrocarbon melting at 63° and an oxime melting at 50° . The higher melting fractions gave a greater proportion of oxime, and since the products from all the fractions appeared to be similar they were united, and in this way there were obtained 4.5 g. of crude hydrocarbon and 2.2 g. of crude oxime. The latter was dissolved in 100 cc. of warm glacial acetic acid and cooled on ice. 0.44 g. of hydrocarbon crystallised out in fine needles. These were filtered off, and to the warmed filtrate an equal volume of warm absolute alcohol was added. On cooling, the major part of the oxime came out as fine flakes. These were dissolved in 30 cc. of warm glacial acetic acid and 0.1 g. of crude hydrocarbon separated by cooling as before. The filtrate was evaporated to dryness *in vacuo*, dissolved in ether, the ethereal solution washed to remove traces of acetic acid and again evaporated to dryness. Yield of oxime 1.5 g.; m.p. $50-51^{\circ}$.

Analysis. 0.0915 g. substance gave 0.2682 g. CO_2 , 0.1050 g. H_2O .

0.1728 g. substance gave 5.0 cc. of moist nitrogen at 18° and 752 mm.

Found: C, 79.91 %; H, 12.88 %; N, 3.29 %.

$\text{C}_{29}\text{H}_{58}\text{NOH}$ requires: C, 79.63 %; H, 13.50 %; N, 3.21 %.

The ketone was regenerated from the oxime by boiling for some hours in alcohol saturated with hydrochloric acid. By repeated crystallisation from benzene-alcohol, there was obtained 0.7 g. of ketone crystallising in thin glistening plates. m.p. $80.5-81^{\circ}$; setting point $80.5-80^{\circ}$.

Analysis. 0.0832 g. substance gave 0.2487 g. CO_2 , 0.1043 g. H_2O .

Found: C, 82.45 %; H, 13.93 %.

$\text{C}_{29}\text{H}_{58}\text{O}$ requires: C, 82.37 %; H, 13.87 %.

The melting point of this substance suggested that it was palmitone, and accordingly a sample of this ketone was synthesised from pure palmitic acid by Kipping's [1890] method. It melted at $82.2-82.5^{\circ}$ and set crystalline at $81.7-82^{\circ}$, confirming the value (82°) given by Kipping and not that (76°) quoted by Saville and Shearer [1925]. A mixture of this palmitone with the cabbage ketone melted sharply at $75.8-76.2^{\circ}$ and set crystalline at $75.5-74.5^{\circ}$, showing that the two ketones were not identical. Mr Piper then kindly submitted the cabbage ketone to X-ray analysis and reported as follows. "Main spacing 38.8 Å. The melted specimen showed only side spacings, but when crystallised and rubbed gave a perfect photograph. The even orders 2 and 4 are just visible, the odd orders 1, 3, 5, 7, 9 give the above spacing. The ketone contains 29 carbon atoms and is probably $\text{C}_{14}\text{H}_{29}\cdot\text{CO}\cdot\text{C}_{14}\text{H}_{29}$, since the oxygen atom appears to be half-way along the chain. The chain is so long however that the possibility of the ketone being $\text{C}_{15}\text{H}_{31}\cdot\text{CO}\cdot\text{C}_{13}\text{H}_{27}$ is not excluded." Both these ketones were accordingly synthesised. The

synthetic di-*n*-tetradecyl ketone melted at 80–80·5° and set crystalline at 79°. Mixed with the cabbage ketone it melted at 80·5–81° and set crystalline at 79°. The synthetic palmityl myristyl ketone melted at 74–74·5°.

Reviewing our experience on the isolation of these two substances from the crude hydrocarbon fraction, there is no doubt that much time and trouble would have been saved if the product obtained by distillation after the initial saponification had been oximated and the oxime removed as described above. The long fractionation actually carried out however has served to show that the chief constituents are nonacosane and di-*n*-tetradecyl ketone, and that they are present in the ratio of three of the former to one of the latter. Other products are present, but they appeared in fractions too small to warrant the hope that pure compounds could be isolated from them.

Synthesis of di-n-tetradecyl ketone, $\text{CH}_3\cdot(\text{CH}_2)_{13}\cdot\text{CO}\cdot(\text{CH}_2)_{13}\cdot\text{CH}_3$.

Kipping's [1890] method for the preparation of palmitone was used. 2 g. of pentadecylic acid (M.P. 52°), for which we have to thank Prof. W. E. Garner, were heated in a small beaker in a metal-bath at 205–210° and phosphorus pentoxide was added slowly in small portions with constant stirring. The reaction was complete in about 5 minutes, when the molten mass was poured into water to decompose the excess of phosphorus pentoxide. Sodium hydroxide was then added to convert the excess pentadecylic acid to soap and the mixture raised to the boiling point. On cooling, the ketone and other resinous products solidified at the surface as a waxy cake, which was removed and washed in the usual way. The ketone was separated from the other products by repeated extraction with small volumes of hot 90 % alcohol. The crude ketone was dissolved in 90 % alcohol, and the solution boiled with charcoal and filtered. On cooling the ketone separated in thin glistening plates. Yield 0·2 g.; M.P. 78·5–79°. Recrystallised twice from benzene-alcohol it melted sharply at 80–80·5°, and with a nucleus set crystalline at 79°.

Analysis. 4·920 mg. substance gave 14·840 mg. CO_2 , 6·05 mg. H_2O .

Found: C, 82·30 %; H, 13·67 %.

$(\text{C}_{14}\text{H}_{29})_2\text{CO}$ requires: C, 82·47 %; H, 13·74 %.

Synthesis of palmityl myristyl ketone, $\text{CH}_3\cdot(\text{CH}_2)_{14}\cdot\text{CO}\cdot(\text{CH}_2)_{12}\cdot\text{CH}_3$.

We have to thank Prof. R. Robinson for suggesting this synthesis, which is similar to that of lactarinic acid, 6-ketostearic acid [Robinson and Robinson, 1925].

Ethyl α -dodecylacetoacetate. Ethyl laurate, obtained by the fractional distillation of the ethyl esters of the fatty acids of coconut oil, was reduced to lauryl alcohol by means of sodium and dry ethyl alcohol at 130°. The alcohol was converted to dodecyl iodide by heating with iodine and red phosphorus. Sodium (1·8 g.) was dissolved in 30 cc. absolute alcohol, 15 g. of ethyl acetoacetate were added and finally 22·5 g. dodecyl iodide. The mixture was boiled

gently for $3\frac{1}{2}$ hours, cooled, poured into water and the mixture extracted with ether. The oil resulting from evaporation of the solvent was fractionally distilled *in vacuo*, and the higher boiling fraction again distilled. The fraction (12.5 g.) collected at $184\text{--}187^{\circ}/8$ mm. analysed as follows:

0.1380 g. substance gave 0.3667 g. CO_2 , 0.1442 g. H_2O .

Found: C, 72.44 %; H, 11.61 %.

$\text{C}_{18}\text{H}_{34}\text{O}_3$ requires: C, 72.50 %; H, 11.41 %.

Palmityl myristyl ketone. 0.9 g. sodium was emulsified under toluene, washed with dry ether and then suspended in 75 cc. of dry ether. 12.1 g. of ethyl α -dodecylacetoacetate dissolved in dry ether were added and the mixture slightly warmed until all the sodium had dissolved. It was then cooled on ice and 12.2 g. palmityl chloride in 20 cc. dry ether were added. The mixture was allowed to stand for 1 hour and then boiled for 10 minutes. The sodium chloride was removed by washing with water and the ether removed by evaporation. The resulting ethyl $\alpha\alpha$ -dodecylpalmitylacetoacetate was an oil which readily crystallised on standing. The product was dissolved in 300 cc. of cold alcohol and as much 5 % aqueous potassium hydroxide added as possible without causing precipitation (14 cc.). After standing for 5 days to effect the slow hydrolysis of the carbethoxy-group, sufficient strong aqueous potassium hydroxide was added to make the concentration 5 %, and the solution boiled for 2 hours. The hot solution was then poured into an excess of boiling water, and after cooling the ketone was removed by extraction with ether. The ethereal solution was concentrated to about 180 cc. and 2 volumes of acetone were added. The palmityl myristyl ketone was almost completely precipitated. It was redissolved in hot acetone and the solution clarified by boiling with charcoal. On cooling, the ketone separated in fine silky plates. Yield 4.5 g.; m.p. $73\text{--}74^{\circ}$. Recrystallised twice from equal volumes of alcohol and benzene, it was obtained in large lustrous plates melting at $74\text{--}74.5^{\circ}$ and setting crystalline at 73° . The melting point was unchanged on further recrystallisation.

Analysis. 4.892 mg. substance gave 14.770 mg. CO_2 , 6.03 mg. H_2O .

Found: C, 82.33 %; H, 13.70 %.

$\text{C}_{15}\text{H}_{31}\cdot\text{CO}\cdot\text{C}_{13}\text{H}_{27}$ requires: C, 82.47 %; H, 13.74 %.

After the removal of the palmityl myristyl ketone, the acetone-ether filtrate was evaporated to dryness and submitted to steam distillation. The distillate was filtered, and from the residue, after crystallisation from aqueous acetone, there was obtained 2.7 g. of methyl tridecyl ketone; m.p. $38.5\text{--}39^{\circ}$.

We wish to express our thanks to Mr S. H. Piper, of the University of Bristol, for the interest he has taken in this work and for submitting a number of specimens to X-ray analysis. One of us (A. C. C.) is indebted to the Royal Society for a grant from which part of the expenses of this research was defrayed.

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