

# CCLVIII. THE ISOLATION OF *n*-OCTACOSANOL FROM WHEAT WAX<sup>1</sup>.

By ALFRED POLLARD, ALBERT CHARLES CHIBNALL,  
AND STEPHEN HARVEY PIPER.

*From the Biochemical Department, Imperial College of Science and Technology, South Kensington, and the Wills Physical Laboratory, University of Bristol.*

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IN continuation of the research dealing with the attempted isolation of the longer-chain primary alcohol constituents of waxes, an account is now given of the preparation from the blades of wheat (*Triticum vulgare*), of *n*-octacosanol.

Purification of the alcohol so that it could be used as a standard for our X-ray and m.p. data has proved extremely troublesome but was eventually effected in quite a simple way by repeated recrystallisation at 37° from acetone. An account, however, of some of our earlier abortive attempts at purification is given in some detail, not only because the methods employed have been used by other workers whose results we shall have occasion to criticise in later papers, but also because they illustrate very clearly the significance of the "heating transition temperature" in judging the purity of a paraffin and hence of the alcohol from which it has been derived. In the present case it has been possible to show quite definitely that the *n*-octacosanol when first isolated from the wax contains not more than about 1 % of impurity, consisting of at least three other alcohols.

## EXPERIMENTAL.

*Preparation and treatment of the wax.* Young wheat at the stage just before the appearance of ears was cut about one foot above ground, and the blades air-dried in a room at 40°. The dried and powdered material (14 kg.) was extracted with ether, and from the material thus obtained (400 g.) the crude wax (62 g.) was prepared by the method of Pollard *et al.* [1931]. The yield was therefore 15.5 % of the ether extract or 0.44 % of the dried wheat. On saponification the wax gave 40 g. of unsaponifiable material, which was a hard yellow wax, 12.4 g. of crude fatty acids and some amorphous material which appeared to consist chiefly of chlorophyll degradation products.

As a preliminary experiment had shown that the unsaponifiable wax when treated by the phthalate method of Chibnall *et al.* [1931] gave only a primary alcohol m.p. 82–83° and a small amount of paraffin, secondary alcohols and ketones being absent, the main bulk of the material was treated in the following way to obviate the labour and losses which always attend the phthalate treatment. The material was dissolved in warm chloroform which was poured into a dish and left exposed to the air. On evaporation of the solvent a yellow friable powder was obtained, which was then shaken for ten minutes at room temperature with light petroleum (b.p. 40–60°). Twelve successive extractions removed

<sup>1</sup> The melting-points recorded in this paper were obtained by the method described by Piper *et al.* [1931] and are corrected.

all material (10 g.) readily soluble in this solvent, leaving 30 g. of insoluble crude primary alcohol.

*Constitution of the primary alcohol.* The crude primary alcohol was crystallised from carbon disulphide to remove the last traces of paraffin, giving 26.6 g. of white granular crystalline powder, m.p. 82–82.5°. 26 g. were acetylated by boiling gently for 16 hours with acetic anhydride (200 cc.) and fused sodium acetate (20 g.). The dark brown solution was poured into water and crushed ice and the mixture stirred for 15 minutes. The brown powder was collected, again stirred with iced water and finally dissolved in benzene-methyl alcohol, leaving a tarry residue. From the solution (charcoal) 27.5 g. of crystalline acetate were obtained, m.p. 63–64°. This material was fractionally distilled *in vacuo* from a Willstätter flask of 100 cc. capacity. The temperature of the metal-bath was about 280° and several fractions were collected at 185–195°/0.03 mm., the melting-points of which ranged from 63.5° to 64.5°. The higher-melting fractions were then collected and redistilled. This operation was repeated many times, but in each case the first two or three fractions melted below 64°. It was then found that if these lower-melting fractions were recrystallised twice from light petroleum (b.p. < 40°) the melting-point in each case rose to 64.4–64.6° suggesting that at the bath temperature necessary to distil the acetate a small amount of decomposition had occurred, with the formation of acetic acid and of  $\Delta^1$ -octacosene, which would be very soluble in cold petroleum. The distillation products were finally collected into four fractions, as shown in Table I, fraction 1 consisting of the

Table I. *Fractionation of the wheat alcohol acetate.*

Fraction no.	Acetate		Alcohol M.P. (° C.)	Paraffin M.P. (° C.)	Acid M.P. (° C.)
	Wt. (g.)	M.P. (° C.)			
1	4.1	64.4–64.6	82.6–82.9	61.3–61.5	90.1–90.5
2	5.7	64.4–64.6	82.6–82.9	61.3–61.5	—
3	8.5	64.3–64.6	82.6–82.9	61.3–61.5	90.0–90.5
4 (residue)	6.7	64.8–65.0	82.2–82.7	61.6–61.8	88.7–89.2

original low-melting samples which as mentioned above had been subsequently recrystallised from light petroleum. The corresponding alcohols were recovered by saponification and were recrystallised repeatedly from acetone and benzene-alcohol until the melting-points were constant.

Fractions 1 to 3 all melted at 82.6–82.9° suggesting that they were of uniform composition, whereas fraction 4, from the undistilled residue melted slightly lower, at 82.2–82.7°. The X-ray photograph of fraction 2 gave (Series B. 214) seven orders measuring 62.0 Å. and of fraction 3 (Series B. 160) 11 orders measuring 62.24 Å. with faint upper spacings measuring 75.46 Å. These results suggested that fractions 1–3 were practically pure *n*-octacosanol, and that the residue (4) contained a small amount of higher homologues.

A sample of each alcohol was then reduced *via* the iodide to the paraffin [Pollard *et al.*, 1931], which was treated with sulphuric acid at 130° until no further darkening of the acid occurred. After washing with water and crystallisation from benzene-alcohol, the resulting product was extracted with cold light petroleum (b.p. 40–60°). As is usually the case with paraffins prepared from long-chain alkyl iodides in this way a very small amount of white amorphous material remained insoluble. The light petroleum was removed by evaporation and the paraffin crystallised from benzene-alcohol.

The melting- and setting-points and transition temperatures of the four paraffins are given in Tables I and II. It will be seen that the distilled fractions (1-3) have melting- and setting-points identical with pure synthetic *n*-octacosane, but that the transition temperatures on heating are 2-3° lower.

Table II.

Fraction no.	Heating transition-point °C.	M.P. °C.	S.P. °C.	Cooling transition-point °C.	Crystal spacings in Å.			Orders measured
					Series no.	A	C	
1	54.8-55.5	61.3-61.5	61.1	53.4	—	—	—	—
2	55.0-55.5	61.3-61.5	61.1	53.4	—	—	—	—
3	54.6-55.0	61.3-61.5	61.1	53.3	B. 162	37.6	—	5
4	53.6-54.0	61.5-61.7	61.3	52.1	—	—	—	—
3a	54.6-55.0	61.3-61.5	61.1	53.2	B. 189	37.79	—	7
3b	54.5-55.0	61.3-61.5	61.1	—	B. 190	37.79	—	7
3c	54.5-55.0	61.3-61.5	61.1	—	B. 191	37.74	—	8
3d	55.0-55.5	61.3-61.5	61.1	53.3	B. 200	—	33.16	—
5	57.0-57.4	61.3-61.5	61.1	54.0	B. 261	—	33.21	15
Synthetic C <sub>28</sub> H <sub>58</sub>	57.0-57.4	61.3-61.5	61.1	54.0	C. 114	—	33.4	8
90% C <sub>28</sub> H <sub>58</sub> + 5% C <sub>26</sub> H <sub>54</sub> + 5% C <sub>30</sub> H <sub>62</sub>	54.0-54.4	61.3-61.5	61.2	—	B. 70	38.0	—	5
98% C <sub>28</sub> H <sub>58</sub> + 1% C <sub>26</sub> H <sub>54</sub> + 1% C <sub>30</sub> H <sub>62</sub>	54.5-55.0	61.3-61.5	61.1	53.3	B. 168	—	33.25	6
99% C <sub>28</sub> H <sub>58</sub> + 1% (Equimolar C <sub>26</sub> H <sub>54</sub> + C <sub>30</sub> H <sub>62</sub> + C <sub>32</sub> H <sub>66</sub> )	55.0-55.5	61.3-61.5	61.1	53.4	B. 212	37.9	—	8
98% C <sub>28</sub> H <sub>58</sub> + 2% above triple mixture	54.0-55.0	61.3-61.6	61.1	53.0	B. 213	37.7	—	5

To obtain if possible a purer sample of *n*-octacosanol the alcohol fraction 3 was passed through the phthalate treatment to remove any trace of secondary alcohol, ketone or naturally occurring paraffin. The m.p. of the resulting alcohol however was unchanged, but the X-ray photograph (Series B. 192) showed 9 orders in the short form, measuring 62.58 Å. with no trace of the longer form, which only appears at room temperature if impurity is present or when the temperature is kept above the transition-point. The derived paraffin had the same m.p., s.p. and transition temperatures as before. The latter was accordingly fractionated by means of cold light petroleum, but the three fractions finally collected (3a, 3b, 3c) showed no improvement in transition temperatures, and all gave crystal spacings (Series B. 189-191) in the A form, 37.8 Å. and were therefore impure. They were therefore united and distilled *in vacuo*. Data concerning the material thus obtained are given in Table II, 3d. It will be seen that there is an improvement of only 0.5° in the transition temperature, but that the crystal spacing now appears in the short, C form, indicating that some contamination had been removed.

Now this persistently low transition temperature for the paraffin and the difficulty of obtaining the crystal spacing in the short form might suggest—from the data for mixtures of C<sub>26</sub>H<sub>54</sub> and C<sub>28</sub>H<sub>58</sub> given in a former paper [Piper *et al.*, 1931]—that the paraffin was C<sub>28</sub>H<sub>58</sub> with either 10% of C<sub>26</sub>H<sub>54</sub>, or 5% of C<sub>30</sub>H<sub>62</sub>, but the melting-point definitely excludes such a possibility. C<sub>28</sub>H<sub>58</sub> with 1% of both C<sub>26</sub>H<sub>54</sub> and C<sub>30</sub>H<sub>62</sub> gives too high a transition temperature on heating and a good X-ray picture in the short form. On the other hand 5% of each of these latter paraffins gives a close approximation to the paraffin from the wheat alcohol. This result was extremely puzzling for during a prolonged fractionation of the derived paraffin referred to above we were unable to effect any displacement of the m.p. and s.p., each fraction, when melted alongside a sample of synthetic *n*-octacosane, giving parallel melting- and setting-points. Yet the transition temperature on heating was always about 2° lower than that of the synthetic paraffin. Had there been 5% or even 2% of impurity in the form of homologues present in this paraffin our experience leads us to believe that we

could have readily obtained a shift in the melting-point by fractionation with cold petroleum.

To check the transition temperature of our synthetic *n*-octacosane we compared it with a second sample prepared from highly pure *n*-tetradecyl iodide by the method of Backmann and Clark [1927]. In a former paper [Piper *et al.*, 1931] this sample was stated to have the correct m.p. and s.p. but to give an inferior X-ray picture. We have since found that the material can be readily purified by extraction at room temperature with low-boiling petroleum, which separates a small amount of insoluble impurity. This new paraffin gave an X-ray picture with 8 orders and a spacing of 33.27 Å. (Series B. 211) and had the same transition temperature on heating (57–57.4°) as the earlier sample of *n*-octacosane.

The X-ray pictures of all the samples of paraffin from the wheat alcohol were so unlike any of those of any even number paraffin containing a small amount of one or two other paraffins which we had yet made, that we decided to prepare some mixtures containing small amounts of three other paraffins, *e.g.* C<sub>28</sub>H<sub>58</sub> with 1 % and with 2 % of an equimolar mixture of C<sub>26</sub>H<sub>54</sub>, C<sub>30</sub>H<sub>62</sub> and C<sub>32</sub>H<sub>66</sub>. Data for the melting-points, transition temperatures and crystal spacings are given in Table II. Only 5 poor orders in the long form were given in both cases, and the picture given by the 1 % mixture approached nearest to, but was markedly inferior to, those given by the paraffin fractions from the wheat alcohol. The transition temperature on heating was not easily observed, but was definitely lower than that of the latter paraffins. Our investigation of the paraffins derived from fractions 1 to 3 of the wheat alcohol therefore suggested that they are *n*-octacosane with less than 1 % impurity made up of at least three other paraffins, and that consequently the alcohol was *n*-octacosanol with less than 1 % admixture of three other alcohols.

Much material and labour were expended in trying to obtain this alcohol chemically pure by repeated fractionation from the usual mixed solvents. The m.p. remained constant at 82.7–83°, and the heating transition temperature of the derived paraffin about 2–3° low. Furthermore the acid given on oxidation, which melted at 90.3–90.5°, gave an X-ray photograph (Series B. 236) with 11 orders in the B form measuring 66.73 Å. and 9 orders in the C form measuring 60.46 Å., whereas the data which we were slowly accumulating for the higher *n*-fatty acids suggested that a really pure acid should give only the B form.

Purification of the alcohol was finally effected quite simply by repeated recrystallisation from relatively large volumes of acetone, not at room temperature, which had been our usual procedure with this and other solvents, but at 37° in an incubator. 2 g. of the alcohol, m.p. 82.7–83°, were recrystallised three times in this way from 800 cc. of acetone. The yield was 0.8 g., and m.p. 83.2–83.4°, unchanged on further treatment. The X-ray photograph (Series B. 256) showed 30 orders in the B form measuring 62.15 Å.<sup>1</sup> The paraffin prepared *via* the iodide (m.p. 60.8–61.2°) had the correct transition temperature (57–57.4°) as shown in Table II (fraction 5) and gave an excellent X-ray photograph with 15 orders in the low or C form measuring 33.21 Å. It was a highly pure sample of *n*-octacosane, showing that the purification of the alcohol had been effective. Oxidation of the alcohol with chromium trioxide in glacial acetic acid [Pollard *et al.*, 1931] gave *n*-octacosanoic acid, m.p. 90.6–90.9°. Crystallisation at 37° from a large volume of acetone raised the m.p. to 90.8–91.1°, unchanged on further

<sup>1</sup> It is known that a pure long-chain compound of *n* carbon atoms usually gives very strong reflections in the *n*th and (*n*+2)th orders. The X-ray technique has been modified during this work to allow measurement of these high orders, and an improved accuracy in the spacings has thus been obtained.

treatment. The crystal spacing (Series B. 262) was in the B form only, up to 30 orders measuring 66.76 Å. The ethyl ester was purified by distillation (155–165°/0.07 mm.) and melted at 64.3–64.5°, Bleyberg and Ulrich [1931] give the m.p. of their synthetic *n*-octacosanoic acid as 90.3–90.5° and of the ethyl ester as 64.8–65°. We shall deal in a later paper with the constitution of “montanyl” alcohol, which has been considered by various workers to be either *n*-octacosanol or *n*-nonacosanol.

*Naturally occurring wheat paraffin.* The 10 g. of material which were fairly readily removed from the unsaponifiable portion of the wax by means of light petroleum were dissolved in warm carbon disulphide. 2.2 g. of crude primary alcohol, m.p. 81–82° crystallised out on cooling. The mother-liquor was taken to dryness and recrystallised from benzene-alcohol and acetone. 2.6 g. of white crystalline material were then obtained which were shown to be free from alcohols and ketones by appropriate treatment. This substance melted at 65–65.5°, and after treatment with sulphuric acid in the usual way 1.7 g. gave 1.4 g. of paraffin melting at 65.5–66°. As was pointed out in a previous paper [Pollard *et al.*, 1931] dealing with the wax fraction of ryegrass, which contained a paraffin with a similar m.p., earlier workers have considered such paraffins to be *n*-triacontane. As in the case of ryegrass the wheat paraffin was shown without difficulty by fractionation with light petroleum to be a complex mixture whose composition cannot at present be suggested (Table III).

Table III. *Fractionation of the paraffin, M.P. 66°, isolated from wheat.*

Series no.	Heating transition point ° C.	M.P. ° C.	Crystal spacing Å.	Number of order of reflection
B. 179	49 –50	61 –61.5	38.9	4
B. 178	51.5–52.5	62.8–63.3	39.6	6
B. 177	54 –55	64.5–65.0	40.6	6
B. 176	58 –59	65.2–65.7	41.1	6
B. 175	59 –60	67 –67.5	43.0	5

#### SUMMARY.

The principal component of the wax from blades of young wheat is a long-chain primary alcohol which has been identified as *n*-octacosanol (m.p. 83.2–83.4°) by reduction to *n*-octacosane (m.p. 61.3–61.5°) and by oxidation to *n*-octacosanoic acid (m.p. 90.8–91.1°). The purity of all three products has been confirmed by X-ray analysis.

The wax also contains mixed fatty acids, the composition of which has not yet been determined, and a paraffin, m.p. 66°, which has been shown to be a complex mixture.

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