

CCLVII. THE ISOLATION OF *n*-TRIACONTANOL FROM LUCERNE WAX¹.

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In a previous paper [Chibnall *et al.*, 1931] dealing with the metabolism of paraffins in the plant the need for gaining some insight into the constitution of the mixed primary alcohols which invariably accompany them in plant waxes was emphasised. We have investigated many such mixed alcohols during the last five years, but definite evidence of their constitution was at first difficult to obtain because their components—higher primary alcohols containing 26 to 36 carbon atoms—had not then been synthesised, so that accurate standards for comparison, based on melting-points and crystal spacings, not only of these alcohols themselves, but also of the corresponding acetates, *n*-fatty acids and ethyl esters, were not available. Much of our work on plant waxes has therefore been deliberately held back pending the possible isolation from certain of them of the unknown primary alcohols in a state of unquestionable purity.

Since the work was in progress *n*-octacosanol, *n*-octacosanoic acid and *n*-triacontanoic acid have been synthesised by Bleyberg and Ulrich [1931] and *n*-triacontanoic acid by Mrs Robinson.

The preparation of pure *n*-hexacosanol from the wax of cocksfoot has already been described [Pollard *et al.*, 1931]. The presence of this alcohol, m.p. 79·8°, was readily recognised by the fact that on oxidation it gave an acid which melted at 87·9°, *i.e.* over 8° higher than the alcohol itself, whereas the mixed primary alcohols which usually occur in waxes give on oxidation acids melting only 2–3° higher, owing to the fact that mixtures of *n*-fatty acids exhibit melting-points depressed many degrees. It was to be expected that this large difference of about 8° between the melting-points of an alcohol and its corresponding acid would hold for the longer-chain primary alcohols for which we were seeking; accordingly we have oxidised a sample of every wax primary alcohol isolated in this laboratory with the hope of finding other cases in which the corresponding acid melted some 8° higher than the alcohol itself. In this we have been more successful than we had dared to hope, and have been able to prepare samples of *n*-octacosanol, *n*-triacontanol and *n*-tetratriacontanol whose purity can be vouched for by data obtained from the derived paraffins. From these alcohols we have been able to obtain the corresponding acetates, *n*-fatty acids and ethyl esters and also similar products containing one more carbon atom. Data concerning the melting-points and crystal spacings of all these substances, and of mixtures of known composition, are fully discussed in another paper.

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

The present paper describes the preparation of *n*-triacontanol from the leaf wax of lucerne (*Medicago sativa*). This wax was first investigated at an early stage in our research when we were seeking for possible sources of ketones corresponding to the 15-nonacosanone isolated by Channon and Chibnall [1929]. Jacobson [1911; 1912] claimed to have obtained two ketones, myristone, $C_{27}H_{54}O$, and alfalfone, $C_{21}H_{42}O$, from lucerne (alfalfa) hay. We were unable to substantiate the findings of Jacobson, however, as our analysis showed that the wax was made up of fatty acids, a primary alcohol and a small amount of mixed paraffin. It was not until a later stage in our research that steps were taken to determine the constitution of this primary alcohol, and it is for this reason that the yield of pure *n*-triacontanol finally obtained falls short of the maximum amount present in the wax.

EXPERIMENTAL.

The lucerne was cut about one foot above ground, so that the sample (53 kg.) consisted chiefly of leaf material. The wax was prepared and saponified in a similar way to that described by Pollard *et al.* [1931] for the products from cocksfoot. The gross weight of wax was 49 g., equivalent to 15 % of the ether extract and 0.35 % of the dry weight of the lucerne; from it were obtained 10 g. of fatty acids which were not further characterised and 36 g. of unsaponifiable material. The constituents of the latter were then separated by the phthalate method of Chibnall *et al.* [1931].

Primary alcohol. 23 g. of unsaponifiable material gave 22.6 g. of insoluble sodium salt of a primary alkyl phthalate, which was thoroughly washed in the usual way with alcohol and ether. On hydrolysis with sodium ethoxide in benzene-alcohol it gave 18 g. of crude primary alcohol, m.p. 85°. 10 g. of this product were dissolved in boiling absolute alcohol, clarified with charcoal and the product which separated on cooling again treated twice successively in the same way. The white crystalline material thus obtained was recrystallised twice from one litre of boiling alcohol and then repeatedly from benzene-alcohol and acetone. The yield was 5 g.; m.p. 85.6–85.8°. On oxidation it gave an acid, m.p. 92.7–93.0°, with a crystal spacing (Series B. 164) of 71.5 Å., and on reduction *via* the iodide and treatment with light petroleum a paraffin m.p. 65.6–65.8° with a crystal spacing (Series B. 184) of 35.4 Å. This latter spacing, corresponding to the C form of *n*-triacontane, suggested that the paraffin must be fairly pure, showing that the primary alcohol was *n*-triacontanol. To effect if possible further purification the remainder of the alcohol was converted in the usual way with acetic anhydride into the acetate, which was then extracted repeatedly with cold light petroleum. The products thus obtained all melted within the limits 68.5–68.8°.

The alcohol recovered from the acetate crystallised from ethyl alcohol in rhombic plates with sharp angles and edges. 0.35 g. was recrystallised twice from 200 cc. of acetone at 37°. The melting-point was then constant at 86.3–86.5°, and the crystals gave a very good X-ray photograph (Series B. 259) showing 13 orders in the B or short form measuring 66.53 Å. A sample was reduced *via* the iodide (m.p. 63.8–64.2°) to the paraffin, which was treated in the usual way with sulphuric acid at 130°. The product thus obtained was extracted three times at room temperature with light petroleum (b.p. < 40°). As is usual with paraffins prepared from long-chain alkyl iodides in this way a very small insoluble residue remained. The extracted material was recrystallised from benzene-alcohol. The melting- and setting-points, transition temperatures and crystal spacings are given in Table I, the corresponding data for *n*-triacontane

Table I. *Paraffin obtained from lucerne alcohol.*

Series no.	Transition point on heating	M.P.	S.P.	Transition point on cooling	Crystal spacing in Å. C form
B. 226	60.2–60.6°	65.6–8°	65.4°	58.8°	35.46
Interpolated data	About 61°	65.6–8°	65.4°	58.8°	35.5

being obtained by interpolation from those of a series of synthetic paraffins examined by Piper *et al.* [1931]. From the discussions given in that paper there is no doubt that the paraffin obtained from the lucerne alcohol contains much less than 1 % impurity, so that the alcohol can be considered a pure sample of *n*-triacontanol.

Oxidation of the alcohol (1 g.) with chromium trioxide and glacial acetic acid [Pollard *et al.*, 1931] gave *n*-triacontanoic acid, which was recrystallised three times at room temperature from acetone. The yield was 0.8 g. and the m.p. 92.6–93.0°. Three recrystallisations from 450 cc. of acetone at 37° gave the pure acid, of m.p. 93.6–93.9° and s.p. 93.1°.

Since this work was completed Mrs G. M. Robinson has kindly placed at our disposal a sample of synthetic *n*-triacontanoic acid¹. This melted at 93.7–94° by the method used in this laboratory. Both acids gave excellent X-ray photographs in the B form (Series B. 238, 235) measuring 71.4 Å., with no trace of the C form, showing that they were pure. Bleyberg and Ulrich [1931] give the m.p. of their synthetic acid as 91.9–92.1°.

Paraffin fraction. After removal of the insoluble sodium salts of the primary alkyl phthalate in the usual way the residual material was taken up in boiling alcohol. On cooling 1.2 g. of impure paraffin crystallised out. As no appreciable amount of material representing sodium salts of secondary alkyl phthalates remained in the mother-liquor, the crude paraffin was next treated with hydroxylamine, but no ketoxime could be separated by the methods employed by Sahai and Chibnall [1932]. These two operations show conclusively that the lucerne wax contains neither secondary alcohols nor ketones. The crude paraffin was then treated with sulphuric acid at 130° until no further darkening of the acid occurred. The resulting product melted at 65.4–65.6° which is close to the m.p. of *n*-triacontane. Three simple extractions with cold petroleum (b.p. < 40°) separated it into fractions whose transition temperatures and melting-points (Table II) show that they are complex mixtures.

 Table II. *Fractionation of the naturally occurring paraffin, M.P. 65.6°, isolated from lucerne.*

Fraction no.	Heating transition-point	M.P.	S.P.	Cooling transition-point
1	56.5–57.2°	64.9–65.1°	64.7°	55.2
2	57.7–58.3°	65.4–65.6°	65.3°	56.2
3	58.4–58.9°	66.0–66.2°	65.8°	56.8

The lucerne wax therefore consists of fatty acids, *n*-triacontanol and a mixture of paraffins whose mean molecular weight corresponds to that of *n*-triacontane. Jacobson [1911; 1912] extracted lucerne (alfalfa) hay with hot alcohol, filtered, evaporated the extract to dryness and took up the residue with ether. On concentration the ethereal solution deposited material which was recrystallised repeatedly from ethyl alcohol, chloroform and methyl alcohol.

¹ Unpublished work. The *n*-triacontanoic acid was obtained by reduction of 13-ketotriacontanoic acid, synthesised from ethyl 11-bromoundecate, ethyl acetoacetate and stearyl chloride by the method outlined in a previous paper [Robinson, 1930].

The product thus obtained, melted at 76.5–77° and represented 0.23 % of the hay. Portions of 0.5–0.8 g. were then treated on the water-bath for 12–15 hours with nitric acid (sp. gr. 1.104), which clarified the material with a loss of only 2.8 % by weight. The waxy cake thus obtained was extracted with hot acetone. The insoluble residue melted at 88.5–88.8° and was soluble in hot chloroform and carbon disulphide. It appeared to give no acetate with acetic anhydride and no acid on oxidation with chromium trioxide in sulphuric acid. It was therefore considered to be a ketone $C_{21}H_{42}O$ and given the name "alfalfone." No evidence for the presence of a keto-group was obtained, other than that on reduction with excess of sodium in alcohol, a product melting at 86.3–86.5° was formed. The material extracted by the hot acetone mentioned above separated as a white voluminous precipitate on cooling. It softened at 68° and melted over the range 74–77°. Because this also did not appear to react with acetic anhydride or chromium trioxide in sulphuric acid it was considered to be a ketone, and from its melting-point was identified as myristone $C_{27}H_{54}O$. On reduction with excess of sodium in alcohol it was stated to give an alcohol which softened at 72°, gave a clear meniscus at 80° and became transparent at 86°. As wax esters of long chain primary alcohols and *n*-acids are saponified only with great difficulty by hot mineral acid there is no doubt that Jacobson's "alfalfone" and "myristone" consisted of unsaponified wax esters of *n*-triacontanol mixed with paraffins, and that the change in melting-point on the assumed reduction with sodium was due to the saponification of these waxes by the sodium ethoxide produced during the reaction. There is no doubt also that the alcohol $C_{20}H_{42}O$ (M.P. 80°, B.P. 395°) isolated by Étard [1892] from lucerne leaves, and named by him "medicagol" was a mixture of *n*-triacontanol and paraffin. We shall deal in a later paper with the constitution of "myricyl" or "melissyl" alcohol, which has been considered by various workers to be either *n*-triacontanol or *n*-hentriacontanol.

SUMMARY.

The principal component of the wax from lucerne leaves is a long-chain primary alcohol (M.P. 86.3–86.5°) which has been identified as *n*-triacontanol by reduction to *n*-triacontane (M.P. 65.6–65.8°) and by oxidation to *n*-triacontanoic acid (M.P. 93.6–93.9°). 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 been determined, and a paraffin, M.P. 65.6° which has been shown to be a complex mixture. No ketone is present, and it is suggested that the "myristone" and "alfalfone" of Jacobson [1911, 1912] were unsaponified wax.

In conclusion we should like to thank Mrs G. M. Robinson for a sample of synthetic *n*-triacontanoic acid.

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