XLIII. THE CONSTITUTION OF COCCERIN.

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INTRODUCTION¹.

LIEBERMANN [1885] showed that boiling benzene extracted an alcohol-insoluble wax which he called coccerin from the cochineal insect (*Coccus cacti*). By saponification with strong alcoholic potash and separation of the acids as calcium soaps he obtained an alcohol, cocceryl alcohol, which melted at 101–104°, and an acid, coccerinic acid, which melted at 92–93°. Two years later Liebermann and Bergami [1887] investigated the structure of these two compounds. Analysis of cocceryl alcohol suggested that it was a C₃₀-alcohol with two oxygen atoms. Acetylation with acetic anhydride in a sealed tube at 170° gave a product, M.P. 48–50°, apparently a diacetate; similar evidence was obtained on benzooylation, and it was concluded that cocceryl alcohol was a dihydric alcohol C₃₀H₆₀(OH)₂. On oxidation with chromic acid it gave an acid M.P. 59–60° which was identified as pentadecanoic acid. Analysis of the coccerinic acid, and of its salts, suggested that it was a hydroxy-acid C₃₁H₆₂O₃, although no evidence for the hydroxyl group was obtained by acetylation or benzooylation. This acid on oxidation was also stated to give pentadecanoic acid, which was identified as its methyl ester, M.P. 66–68°. As coccerin gave by saponification two parts of acid to one of alcohol it was considered to be C₃₀H₆₀(C₃₁H₆₂O₃)₂, in keeping with the dihydric nature of the alcohol.

The wax has been more recently examined by Becker [1931], who recrystallised it many times from benzene and chloroform, thereby raising the M.P. to 104–105°. By saponification with *N*/2 alcoholic potash and separation of the calcium soaps he obtained approximately equal amounts of alcohol, M.P. 102°, and of acid, M.P. 94–95°. His analysis suggested that the alcohol was C₃₂H₆₆O₂, and on acetylation in the usual way with acetic anhydride and fused sodium acetate it gave a monoacetate, M.P. 78–79°. Treatment in a sealed tube with acetic anhydride at 170° gave a mixture from which only the monoacetate could be recovered. His analysis of coccerinic acid agreed better with the formula

¹ The melting-points below 110° (except those of oximes) recorded in this paper were obtained by the method described by Piper *et al.* [1931] and are corrected.

The nomenclature used in this paper is based on the following system, which has recently been made official (permissive) by the International Union of Chemistry [*cf. J. Chem. Soc.* (1931), 1610].

R.CH ₃	R.CH ₂ OH	R.CHO	R.CO ₂ H	R.COCl	R.CONH ₂	R.CH ₂ NH ₂
-ane	-anol	-anal	-anoic acid	-anoyl chloride	-anoamide	-anamine

The number will be placed immediately before the suffix to which it refers, and the C of the CO₂H group will be numbered 1. The system is more logical than the one previously used in publications from this laboratory and will be adopted in future. A. C. C.

$C_{32}H_{64}O_3$, and accepting Liebermann and Bergami's isolation of pentadecanoic acid, he concluded that cocceryl alcohol and coccerinic acid had corresponding structures. Coccerin therefore had the same biological origin as the plant waxes, and its components were formed by a Cannizzaro reaction from two molecules of an aldehyde built up by repeated aldol condensation.

In connection with the research which is being carried out in this laboratory into the metabolism of waxes this cocceryl alcohol was of interest. Since pentadecanoic acid is now known to melt at 54° and its methyl ester at about 19° we were not prepared to accept all Liebermann's conclusions; in particular Becker's failure to obtain a diacetate suggested to us that the second oxygen atom might be ketonic. Clemmensen reduction might therefore provide us with *n*-dotriacontanol, an alcohol which would be extremely useful in connection with the X-ray analytical work which we are undertaking in conjunction with Dr Piper.

Our first preparation of the wax was made from 15 kg. of cochineal by Liebermann's method. By saponifying in strong benzene-alcoholic potash and separating the acids as their calcium soaps there were obtained approximately equal amounts of alcohol and acid, confirming the observations of Becker. The alcohol after many recrystallisations from benzene and alcohol melted at $99-100^\circ$. A typical example of one out of many analyses confirmed the data of both Liebermann and Becker, *viz.* C, 79.5 % and H, 13.2 %. On acetylation with acetic anhydride in the presence of fused sodium acetate we obtained a monoacetate, again confirming Becker's results. X-ray analysis gave a good photograph, 23 orders measuring $90.4\text{ \AA}$. At the time we could not be certain of the chain length, but we were able to deduce two facts: (1) if the chain length were about 32 carbon atoms, then the doubled spacing showed that the alcohol group was attached to the last or penultimate carbon atom; (2) the intensity distribution showed that another polar group was attached at some point in the carbon chain.

Treatment with hydroxylamine readily gave an oxime, m.p. $77.5-79^\circ$, confirming our surmise that the second oxygen atom was ketonic. Clemmensen reduction gave a monohydric alcohol, m.p. 89.7° , and this on reduction *via* the iodide a paraffin. The melting-point and transition temperatures of the latter showed that it was slightly impure *n*-tetratriacontane, and this was confirmed by X-ray analysis, which gave both the A and C spacings, and we know that the presence of the C spacing is specific [Piper *et al.*, 1931]. X-ray analysis of the monohydric alcohol gave a fair picture, 11 orders measuring $90.2\text{ \AA}$. At this stage in our research the melting-points and crystal spacings for the *n*-fatty acids and primary alcohols with more than 26 carbon atoms were not known, but by extrapolating the data obtained by Malkin [1930] from the primary alcohols with 12 to 25 carbon atoms this spacing gave a reasonably good agreement for the primary alcohol with 34 carbon atoms. Cocceryl alcohol was therefore a keto-*n*-tetratriacontanol, which requires C, 80.2 % and H, 13.5 %. These values were appreciably higher than we and the above-mentioned authors had obtained, and it was clear that some oxygen-rich impurity was present, because a small amount of unsaponified wax would not lower the carbon content. Repeated attempts to purify the alcohol and its acetate with various solvents were not very successful.

Another preparation of wax from 15 kg. of cochineal was obtained, and this was submitted to repeated extraction with large volumes of benzene-alcohol (1:5). After the tenth extraction the residue melted sharply at 106° and its weight was only about one-third that of the original wax. This was saponified first

with benzene-alcoholic potash and then with sodium ethoxide, the acids in both cases being converted into the calcium soaps. The cocceryl alcohol thus obtained melted sharply at 100–100.2°, and on analysis gave, within experimental error, the correct figures, viz. C, 80.0 %, H, 13.5 %. Although this procedure undoubtedly removed the impurity it was wasteful, and as we were desirous of collecting a large quantity of both cocceryl alcohol and the acids for further analysis we repeated it with another batch of wax (from 30 kg. of cochineal) at the same time paying attention to the products extracted by the benzene-alcohol, which were finally collected into two fractions. Full details are given in the experimental section. The results show clearly that extraction with hot benzene-alcohol does not *per se* effect very much purification. As will be discussed later, the so-called coccerinic acid of Liebermann is a mixture of *n*-fatty acids and a ketonic acid, so that coccerin is a mixture of true waxes whose solubility in hot benzene-alcohol will naturally depend on the chain-length of the particular acid component. The impurity present is undoubtedly stable to saponification with alcoholic potash, but is converted into water-soluble products by saponification with sodium ethoxide, and it is the omission of this essential step which has led to the misleading conclusions drawn from earlier work.

The purified cocceryl alcohol on Clemmensen reduction gave a monohydric alcohol, M.P. 92.2°, which was shown to be *n*-tetratriacontanol by (1) reduction *via* the iodide to *n*-tetratriacontane, M.P. 72.6–72.8°, and (2) oxidation to *n*-tetratriacontanoic acid, M.P. 98.3–98.5°; the purity of all three products was confirmed by X-ray analysis.

Oxidation of the keto-alcohol with chromium trioxide in glacial acetic acid gave the corresponding ketonic acid in good yield. This was treated with hydroxylamine and the resulting oxime converted into the corresponding amides by heating with concentrated sulphuric acid. These amides were hydrolysed by heating with hydrochloric acid and the products thus obtained were identified as *n*-nonadecanamine, *n*-eicosanoic acid, *n*-tridecane-(1:13)-dicarboxylic acid and 14-aminomyristic acid. From this it follows that the ketonic acid is 15-keto-*n*-tetratriacontanoic acid and that the above products are formed as follows:

Cocceryl alcohol is therefore 15-keto-*n*-tetratriacontanol.

The acids obtained from the three fractions of the wax, the so-called coccerinic acid of Liebermann, showed variations in melting-point suggesting that they were mixtures similar to those met with as components of plant waxes. Analysis for carbon and hydrogen however again showed the presence of excess oxygen above that required for appropriate mixtures of *n*-fatty acids. Each fraction was unaffected by acetylation, showing that the acids were not hydroxy-acids,

as had been suggested by Liebermann. But treatment with hydroxylamine gave a product freely soluble in cold acetone which could be readily separated from an insoluble residue. This soluble product on purification melted in each case at 56–57°, and analysis showed that it was a ketoxime. Hydrolysis regenerated the ketonic acid, which melted at 104·5–105°. This was shown to be a keto-*n*-dotriacontanoic acid by Clemmensen reduction to *n*-dotriacontanoic acid. The position of the ketonic group was determined as before by heating the ketoxime with sulphuric acid to effect a Beckmann transformation. The resulting amides were hydrolysed with hydrochloric acid, and the products thus obtained were identified as *n*-nonadecanamine, *n*-eicosanoic acid, brassylic acid and 12-aminolauric acid. From this it follows that the ketonic acid is 13-keto-*n*-dotriacontanoic acid, and that the above products are formed as follows:

The constitution has been confirmed by synthesis.

The fraction of the acids insoluble in cold acetone was again treated with hydroxylamine to remove any residual ketonic acid and the product finally obtained recrystallised twice from acetone at 37°. This melted at 93·6° and was shown to be *n*-triacontanoic acid.

It will be clear from the above results that the "coccerinic acid" of Liebermann was a mixture, and as correctly the name should be applied to the acid corresponding to cocceryl alcohol, *i.e.* 15-keto-*n*-tetratriacontanoic acid, which has not yet been found in natural products, we recommend that it be abandoned.

This elucidation of the structure of coccerin is of great interest from the point of view of wax metabolism, for not only do the ketonic alcohol and ketonic acid provide a clear case of β -oxidation, but they both contain the group $\text{CH}_3 \cdot (\text{CH}_2)_{18} \cdot \text{CO}$, which corresponds with the group $\text{CH}_3 \cdot (\text{CH}_2)_{18} \cdot \text{CHOH}$ present in *n*-nonacosan-10-ol, a constituent of apple cuticle wax [Chibnall *et al.*, 1931]. Further discussion is reserved for a later paper.

The difficulty which we experienced in the early stages of this research in purifying coccerin and its constituent alcohol and acids has forcibly drawn our attention to two steps in the procedure which we now consider to be essential. In the first place the initial saponification of the wax with alcoholic potash should be followed by saponification with sodium ethoxide. This is a usual precaution if esters of sterols are known to be present, but it is necessary in the present case in order to remove more effectively impurities which are stable to boiling alcoholic potash. In the second place the alcohol and acid liberated by saponification cannot be effectively separated unless the acid is converted into the very insoluble calcium soap. Liebermann adopted this procedure, as also have certain more recent workers, but others have omitted it probably because it has not been generally realised that the potassium or sodium soaps of the

fatty acids with carbon chains longer than 26 become increasingly soluble in hot acetone or hot benzene with increase of chain length. For these two reasons we consider that most, if not all, of the previous work on the constitution of insect waxes is in need of reinvestigation, and it is to be regretted that in most cases the waxes themselves are so difficult to obtain even in small amounts.

This applies especially to the wax of *Pemphigus xylostei*, which has been recently investigated by Schulz and Becker [1931]. The insects were collected over a period of many years and gave 1.9 g. of wax. This was repeatedly crystallised from chloroform-alcohol, and the sharp melting-point, 108–108.5°, to which they call attention, suggests that extraneous material had been successfully removed. The wax was then saponified with benzene-alcoholic potash, the alkali removed by washing with water and the alcohol (pemphigus alcohol) separated from the acid (pemphigus acid) by extraction with hot benzene. The pemphigus alcohol was recrystallised many times from hot alcohol, but the indefinite melting-point (100–105°) shows clearly that the material was still contaminated with a small amount of potassium soap. Carbon and hydrogen analysis of the alcohol and of its acetate (m.p. 75–77°) suggested that it was a C_{34} dihydric alcohol, the higher homologue of cocceryl alcohol. Accepting Liebermann's isolation of pentadecanoic acid after oxidation of the latter they suggest that pemphigus alcohol is 18-hydroxytetratriacontanol. We do not agree with these conclusions. Their analysis of the alcohol (C, 80.0, 80.21; H, 13.46, 13.50°) agrees much better with that of a ketotetratriacontanol (C, 80.2; H, 13.5 %) than with that of a hydroxytetratriacontanol (C, 79.9; H, 13.8 %). The melting-point (75–77°) of their acetate suggests to us the presence of only one acetyl group (*cf.* the acetate of cocceryl alcohol, m.p. 80.9–81.3°); they infer that it is a diacetate from the result of a single analysis for carbon and hydrogen on a very small amount of material. To obtain collateral evidence we have ourselves reduced cocceryl alcohol to 15-hydroxy-*n*-tetratriacontanol, and as we expected, the diacetate has a much lower melting-point (50–51°). Pending reinvestigation of this wax therefore we prefer to consider that pemphigus alcohol is a ketotetratriacontanol, and that in all probability it is identical with cocceryl alcohol. The melting-point of pemphigus acid (101–102°) is too low for a hydroxy- (or keto-) acid with 34 carbon atoms as they suggest, and it is undoubtedly a mixture similar to "coccerinic acid" but containing a small proportion of *n*-fatty acid, so that the melting-point of the pemphigus wax (108–108.5°) should be higher than that of coccerin (106–106.5°).

EXPERIMENTAL.

Preparation of the wax. 30 kg. of cochineal were extracted with boiling benzene by British Drug Houses, Ltd. The resulting extract, from which the main bulk of the solvent had already been removed before it was delivered to the laboratory, was boiled for 15 minutes with an equal volume (5100 ml.) of benzene and the mixture allowed to stand until cold. The product which separated was filtered off and recrystallised from 3 litres of boiling benzene. The weight of crude wax, dried in an air-oven at 90°, was 149 g., and the m.p. indefinite 99–102°.

Separation of the wax into three fractions. The crude wax was first boiled with 2 litres of 95 % alcohol for 6 hours and the mixture filtered hot. The residue melted at 103–104° and the material (2.5 g.) recovered from the solvent at 98–98.5°. The residue was then extracted seven times successively for 1 hour with 2 litres of boiling benzene-alcohol (1:4), the mixtures being filtered hot through a conical filter under slight suction. The residue melted at 105–106°, while the material recovered from the last hot filtrate melted at 100–101°. The total amount of material recovered from these first eight extractions (Fraction 1), weighed 35 g. Seven further extractions with similar amounts of benzene-alcohol were then made. The material recovered from these extracts (Fraction 2) weighed 34.9 g. and melted at 103–103.5°. The final residue (Fraction 3) weighed 62.0 g. and melted at 106–106.5°. A further 9.7 g. of crude wax were recovered from the numerous filter-papers used in the above separation and were neglected.

Saponification of the wax. The treatment of Fraction 3 given below applies equally to Fractions 1 and 2. The wax (62 g.) was dissolved in 1800 ml. of hot benzene, 1200 ml. of 10 % alcoholic potash were added, and the mixture was boiled under reflux for 12 hours. At the end of that period 3 litres of 95 % alcohol containing 160 g. of calcium chloride were added and the boiling continued for another 2½ hours. The mixture was then filtered hot, using a battery of hot-water-jacketed conical filters. The residual calcium soaps were extracted three times successively with 3 litres of boiling alcohol and then with the same volume of boiling acetone. The filtrates were cooled, and the crude cocceryl alcohol which separated was collected. This material (41 g.) was treated five times successively with boiling acetone and filtered hot. The filtrates on cooling gave 24 g. of crude alcohol. The residue insoluble in the hot acetone (12 g.) contained calcium soaps, which in the previous stage must have been rendered soluble in hot alcohol by the excess of potash present, a little unsaponified wax and much unidentified material. The crude alcohol and residue were therefore collected, dissolved in 1200 ml. of hot benzene and boiled under reflux for 3½ hours with sodium ethoxide (20 g. of sodium in 480 ml. of alcohol). 30 g. of calcium chloride in 250 ml. of 95 % alcohol were then added, and the boiling was continued for 2 hours. The mixture was filtered hot, and the insoluble soaps were extracted three times successively with 1 litre of boiling 95 % alcohol and finally with 1 litre of boiling acetone. The combined filtrates on cooling gave 26.5 g. of crude cocceryl alcohol, m.p. 99–100°. This was digested with 3½ litres of boiling acetone and filtered hot. The filtrate on cooling gave 15 g. of alcohol, m.p. 99.6–99.8°. The residue was treated three times successively in the same way, giving a further 4.7 g. of alcohol, m.p. 100.2–100.5°. The ultimate residue of soap weighed 0.8 g., and the acetone mother-liquors on evaporation gave 4 g. of crude alcohol which was neglected.

The calcium soaps from the first saponification were boiled under reflux with 2 litres of glacial acetic acid until the solution was clear and the mixture poured into an excess of cold water. The precipitated acids were collected, the treatment with glacial acetic acid repeated and the product crystallised from acetone. The material thus obtained (25.7 g.) was digested for some time with 3 litres of boiling acetone and filtered hot. On cooling, the filtrate gave 14.1 g. of fatty acid, m.p. 98.2–99°. Three further extractions of the residue gave in all 6.5 g. of fatty acid, m.p. 104–105°. The ultimate residue was neglected, as also were the 3 g. of crude acid recovered from the acetone mother-liquors on evaporation. The gross yield of acids recovered was 23.6 g. and of alcohol 23.7 g., showing a loss of 14.7 g.

Fraction 2 on similar treatment gave 13.1 g. of crude acids and 11.0 g. of crude alcohol, showing a loss of 10.8 g. The crude acids on digestion with hot acetone gave 11.6 g. of material, m.p. 98.0–98.4°, and the crude alcohol 9 g. of material, m.p. 99–100°. Fraction 1 in the same way gave 12.5 g. of crude acid and 11.0 g. of crude alcohol, showing a loss of 11.5 g. The acetone treatment gave 11 g. of acid, m.p. 89–90°, and 9 g. of alcohol, m.p. 100–101°. In all three fractions the major part of the loss occurred during the saponification with sodium ethoxide.

Fractionation of the fatty acids. The two samples of acid from Fraction 1, and the samples from Fractions 2 and 3 were separately treated with hydroxylamine in the following way. The acid (14.1 g.) in 800 ml. of alcohol was boiled under reflux for 8 hours with 6 g. of hydroxylamine hydrochloride and 9 g. of potassium hydroxide. The mixture was then poured into 6 litres of dilute sulphuric acid, the separated material filtered off and washed thoroughly with

water. It was then taken up in 1300 ml. of warm acetone; 300 ml. of ether were stirred in, and the mixture was allowed to stand for some hours at room temperature. 4.35 g. of *n*-fatty acid crystallised out and were filtered off. The other three samples gave in the same way 0.93, 6.35 and 1.8 g. of crystalline *n*-fatty acid, which were further treated as described later.

The acetone-ether mother-liquors were reduced to one-eighth of their original volume and cooled in the ice-chest. The material which separated was filtered off and dissolved in 1 litre of hot acetone. On cooling to room temperature a first crop of crude oxime weighing 6.4 g. was obtained. The mother-liquor was concentrated to 200 ml. and cooled in the ice-chest. A second crop of crude oxime was thus obtained. The latter was repeatedly extracted at room temperature with changes of cold acetone (shaking and centrifuging) until only a small residue remained undissolved. On evaporation of the solvent the oxime was recovered, and the cold extraction was repeated. The oxime (12.75 g.) then crystallised out in rosettes of white needles.

Constitution of the ketonic acid.

Preparation of the ketonic acid from the ketoxime. The ketoxime melted at 56–57°. (Found: C, 75.5; H, 12.5; N, 2.4 %. $C_{32}H_{62}O_2 \cdot NOH$ requires C, 75.4; H, 12.5; N, 2.7 %.) The regeneration of the ketonic acid from the ketoxime was found to be unexpectedly difficult, owing presumably to a small amount of amide formation. The following procedure was tedious and wasteful, and we are hoping that further research will enable us to increase the yield materially. 9.8 g. of ketoxime were warmed on the water-bath with 100 ml. of formaldehyde (40 %), and then 100 ml. of concentrated hydrochloric acid were added with stirring [Lapworth, 1907]. Separation of solid material rapidly took place and appeared to be complete in about 2 minutes. After remaining on the bath for about 15 minutes the mixture was poured into water, filtered and the residue washed with water and then crystallised from acetone. The material, which was recovered in quantitative yield, melted at 99.5–100°. After a prolonged fractionation from acetone at 37° there were finally obtained 1.9 g. of the ketonic acid, m.p. 104.5–105°. (Found: C, 77.9; H, 12.7 %. $C_{32}H_{62}O_3$ requires: C, 77.7; H, 12.6 %.) The lowest-melting fraction obtained melted at 96.4–96.7° and contained about 0.2 % of N (Dumas).

Conversion of the oxime of the ketonic acid into the amides. The oxime (4.4 g.) was heated on the water-bath with concentrated sulphuric acid (25 ml.) for 2½ hours. The brown solution was cooled and slowly poured on to crushed ice, and the precipitated amides were collected and crystallised first from 250 ml. of glacial acetic acid and then from an equal volume of acetone. The product obtained was a faintly pink crystalline powder melting at 102°.

Hydrolysis of the above amides. 2.5 g. of the amides were heated with 12 ml. of concentrated hydrochloric acid in a sealed tube at 180° for 4 hours. The resulting dark brown products were mixed with 100 ml. of water and distilled in a current of steam. No volatile acids were separated. The aqueous mixture was therefore filtered and the insoluble material taken up in hot alcohol and filtered to remove charred products. The filtrate was concentrated to 50 ml. and 2 volumes of ether were added. On standing overnight a mass of thin white crystalline flakes separated, which were filtered off (Fraction A; wt. 0.29 g.). The filtrate was concentrated to 10 ml.; 20 ml. of hot benzene were added and the mixture allowed to stand overnight. In the morning a further 30 ml. of benzene were added with stirring and the white solid which separated was filtered off (Fraction B; wt. 0.38 g.). The mother-liquor was taken to dryness and the

residue dissolved in 60 ml. of hot alcohol. The solution was made alkaline to phenolphthalein with alcoholic sodium hydroxide and allowed to stand overnight. A white powder separated and was filtered off (Fraction C; 0.45 g.). The mother-liquor on evaporation gave Fraction D.

Fraction A. This was crystallised from 40 ml. of hot benzene, and gave 0.26 g. of material which was then repeatedly extracted with ether. The insoluble residue was dissolved in 20 ml. of alcohol and 2 volumes of ether were added. The mass of thin colourless plates which separated overnight was filtered off and washed with ether. The m.p. was indefinite and analysis suggested that the substance was *n*-nonadecanamine hydrochloride. (Found: C, 71.3; H, 13.3; N, 4.2 %. $C_{19}H_{41}N.HCl$ requires C, 71.3; H, 13.2; N, 4.4 %.) For confirmation this amine, which has not hitherto been prepared, was synthesised from pure *n*-eicosanoic acid by the method of Jeffreys [1899]. The synthetic amine and the substance from Fraction A both gave benzoates crystallising from alcohol in fine needles melting sharply at 88° either alone or when mixed. Samples of *n*-octadecanamine hydrochloride and *n*-eicosanamine hydrochloride gave benzoates melting at 88.2° and 91.5° respectively, which were depressed when mixed with the benzoate from Fraction A.

Fraction B. This was extracted several times with boiling benzene and the insoluble residue crystallised from 20 ml. of 2*N* hydrochloric acid. A first small crop of crystals separated in a few hours and was filtered off. These melted at 145° and were found impossible to purify. The filtrate on long standing gave a second crop of material in the form of fine colourless lamellae. These melted at 163°, alone and when mixed with a sample of authentic 12-aminolauric acid hydrochloride prepared by the late Dr Le Sueur. (Found: (micro-Dumas) N, 5.6%. $C_{12}H_{25}O_2N.HCl$ requires: N, 5.6%.) The micro-Kjeldahl method gave a low result, possibly owing to ring formation. The free amino-acid was obtained by dissolving the hydrochloride (18 mg.) in the calculated volume of dilute sodium hydroxide solution. The white crystalline mass which separated on cooling was collected, washed with cold water and dissolved in boiling water (10 ml.) from which it crystallised on cooling in large thin plates. The m.p. was 183° (uncorr.), unchanged when mixed with a sample of authentic 12-aminolauric acid prepared by the late Dr Le Sueur (m.p. 183°). (Found: (micro-Dumas) N, 6.5 %. $C_{12}H_{25}O_2N$ requires: N, 6.5 %.)

Fraction C. This was dissolved in 30 ml. of glacial acetic acid to decompose the soaps and poured into water. The solid which separated was filtered off and crystallised from ice-cold acetone. Three crops were obtained melting at 66°, 72.5° and 70° respectively. The first of these was extracted several times with boiling light petroleum (b.p. < 40°) and the extracts were concentrated to 10 ml. The crystalline material which separated melted at 73°. This and the second and third crops mentioned above were collected (0.15 g.) and recrystallised twice from 10 ml. of 90 % alcohol. The final product melted at 74–74.2°, and analysis showed that it was *n*-eicosanoic acid. (Found: C, 77.2; H, 12.9 %; mol. wt. by titration 313. $C_{20}H_{40}O_2$ requires: C, 76.9; H, 12.9 %; mol. wt. 312.) A mixed m.p. of 74.4–74.8° was obtained with a sample of synthetic *n*-eicosanoic acid, m.p. 75.0–75.2°, which had been purified by fractional distillation of the ethyl ester. In a mixed melt with synthetic *n*-heneicosanoic acid, m.p. 74.2–74.6° the m.p. was depressed to 70.5°.

Fraction D. This was treated with ether and allowed to stand overnight. The solid which separated was filtered off and the ethereal filtrate extracted with water. The solid was dissolved in this aqueous extract, which was then acidified and again extracted with ether. The ethereal solution yielded 0.23 g.

of solid, which was extracted repeatedly with small quantities of light petroleum (B.P. < 40°). The residue was recrystallised twice from benzene, then from acetone (charcoal) and again from benzene. The product (0.09 g., M.P. 108°) was dissolved in 150 ml. of boiling water, which was filtered hot. Fine white needles separated on cooling (M.P. 111.5°). These were collected, dissolved in 30 ml. of boiling absolute alcohol and titrated hot to phenolphthalein with alcoholic sodium hydroxide. After standing some days small plates of the sodium soap crystallised out. These were filtered off and dissolved in 50 ml. of boiling dilute hydrochloric acid. The crystals which separated on cooling were shown to be brassylic acid. (Found: C, 64.3; H, 10.0 %; mol. wt. by titration (dibasic acid) 250. $C_{13}H_{24}O_4$ requires: C, 63.9; H, 9.9 %; mol. wt. 244.) The M.P. was 113°, unchanged when mixed with authentic brassylic acid (M.P. 113.5°).

Synthesis of 13-keto-n-dotriacontanoic acid. The method of Robinson [1930 and unpublished data] was used. Ethyl 11-bromoundecanoate was condensed with ethyl sodioacetoacetate to give ethyl 2-acetylbrassylate. Ethyl sodio-2-acetylbrassylate (from 4 g. of the ester) in anhydrous ether was added slowly to *n*-eicosanoyl chloride (from 2.8 g. of the acid) in anhydrous ether and the mixture allowed to stand overnight. The next morning it was boiled to complete the reaction, the ether removed and the condensation product extracted with 50 ml. of alcohol. 3 litres of 3 % alcoholic (95 %) sodium hydroxide were then added, and the mixture shaken for 10 hours on two successive days. Half the alcohol was then removed by evaporation and the remainder boiled under reflux for 1 hour. After cooling in the ice-chest the precipitated soaps were filtered off, decomposed by boiling with aqueous hydrochloric acid and the free acids crystallised first from acetone and then from glacial acetic acid. The yield of crude acid was 1.1 g., M.P. 99.5–100.5°. To remove residual *n*-eicosanoic acid the material was recrystallised three times successively from acetone at 37°. The melting-point was 104.5–105°, unchanged when mixed with the cochineal acid. (Found: (Schoeller) C, 77.7; H, 12.6 %. $C_{32}H_{62}O_3$ requires: C, 77.7; H, 12.6 %.) The oxime was prepared in the usual way and crystallised repeatedly from acetone. The melting-point was 58–59°, and 57–58° when mixed with the oxime of the cochineal acid, which melted at 56–57°.

Constitution of the n-fatty acids.

The four samples of *n*-fatty acids obtained during the fractional crystallisation of the ketoxime were collected and 12 g. again treated with hydroxylamine as before. 1.5 g. of crude oxime and 9 g. of *n*-fatty acid, M.P. 92.8–93°, were obtained. The *n*-fatty acid was recrystallised twice from acetone at 37°, when the melting-point was raised to 93.4–93.6°, unchanged on further treatment. It was *n*-triacontanoic acid. (Found: C, 79.5; H, 13.3 %; mol. wt. by titration 454, 453. $C_{30}H_{60}O_2$ requires: C, 79.6; H, 13.4 %; mol. wt. 452.5.) The mixed melt with pure *n*-triacontanoic acid (M.P. 94°) was 93.6–93.8°. The X-ray photograph (Series B, 271) gave 32 orders in the B form measuring 71.1 Å., as against 71.4 Å. standard. There was a trace of the C spacing in the 3rd order showing that the acid probably contained less than 1 % either of higher *n*-fatty acids or of the keto-acid which had escaped oximation. This is the first recorded isolation from natural sources of *n*-triacontanoic acid as distinct from “melissic acid,” which melts at 90–91° and has been considered by various workers to be a normal or *iso*-fatty acid with either 30 or 31 carbon atoms. We shall deal with the constitution of this acid in a later paper.

Constitution of cocceryl alcohol.

The samples of cocceryl alcohol obtained from the three fractions mentioned above were purified by treatment with acetone in the following way. The material (37.7 g.) was stirred with 1200 ml. of acetone at 40° for a short time and filtered hot. The residue was recrystallised from boiling acetone and the product thus obtained again treated with 1200 ml. of acetone at 40°. After the fourth repetition of this operation the m.p. of the final residue (26 g.) was constant at 100.5–100.7°. Treatment of the acetone mother-liquor gave several fractions melting between 99° and 100° which have not yet been further purified.

Properties of cocceryl alcohol. The pure alcohol crystallises from most organic solvents, and particularly from acetone at 37°, in small colourless rhombic leaflets which melt sharply at 100.5–100.7° and set with a nucleus in long needles at 99.7°. (Found: C, 80.0, 80.0; H, 13.5, 13.4 %. $C_{34}H_{68}O_2$ requires: C, 80.2; H, 13.5 %.) It is insoluble in all organic solvents in the cold, is slightly soluble in boiling ether and low-boiling petroleum and is readily soluble in boiling benzene or boiling acetone.

Treatment with acetic anhydride and fused sodium acetate gave the *monoacetate*, m.p. 80.9–81.3°, which is soluble in ether and to a small extent in cold acetone. (Found: C, 78.3; H, 12.7 %. $C_{36}H_{70}O_3$ requires: C, 78.5; H, 12.8 %.) To find out if the presumed diacetate of Liebermann and Bergami [1887] was obtained through enolisation of the ketonic group we prepared the acetate according to their directions. 1 g. of the alcohol was heated with 0.5 g. of acetic anhydride in a sealed tube at 170° for 2 hours. The resulting product crystallised from ether-acetone in fine rhombic plates melting at 80.9–81.2° and was the monoacetate.

The *oxime*, prepared in the usual way and crystallised from alcohol, melted at 74.5–75°. (Found: C, 77.4; H, 13.5; N, 2.5 %. $C_{34}H_{68}O.NOH$ requires: C, 77.9; H, 13.3; N, 2.7 %.) It is readily soluble in cold ether and sparingly so in cold acetone.

Reduction of cocceryl alcohol to n-tetratriacontanol. 17.7 g. of cocceryl alcohol were reduced by Clemmensen's method [Le Sueur, 1915] using 160 g. of amalgamated zinc (2 portions) and 160 ml. of concentrated hydrochloric acid (changed six times). The reaction was allowed to proceed for 60 hours, when the acid was diluted with water and the reduced alcohol, together with the residual zinc, filtered off. The mixed product was treated with a large volume of boiling acetone, which was filtered hot. On cooling the filtrate gave 14.8 g. of reduced alcohol, m.p. 88.5–89.0°. A second treatment with hot acetone gave a further 3.2 g., m.p. 90.9–91.3°. The lower-melting fraction was recrystallised twice from acetone at 37° which raised the melting-point to 91.2–91.5°. The products were then combined and 10 g. acetylated with 12.8 g. of fused sodium acetate and 300 ml. of acetic anhydride. The acetate was washed with water, crystallised from acetone and then distilled. The bath temperature was 305°, and 6 fractions were collected at 220–235°/0.004 mm. On crystallisation from acetone they all melted at 75.5–75.8°. The collected material (7.7 g.) was saponified and the resulting alcohol crystallised twice from 4 litres of benzene-acetone (1:2) at 37° when the melting-point was constant at 91.9–92.2°. The yield was 5 g. (Found: C, 82.6; H, 14.2 %. $C_{34}H_{70}O$ requires C, 82.5; H, 14.3 %.) The large colourless rhombic plates obtained from acetone gave an excellent X-ray photograph (Series B, 266); up to 36 orders measuring 74.64 Å. in the B or short form, with no trace of the A or long form. The purity of the alcohol was checked by reduction *via* the iodide to *n*-tetratriacontane [Pollard *et al.*, 1931]. After treat-

ment with sulphuric acid this had M.P. 72.6–72.8°; S.P. 72.5°, and heating and cooling transition temperatures of 69.2–4° and 68.5° respectively, in excellent agreement with those of the pure synthetic paraffin [Piper *et al.*, 1931]. *n*-Tetra-triacontanoic acid was prepared by oxidising the alcohol (0.5 g.) with chromium trioxide in glacial acetic acid in the usual way [Pollard *et al.*, 1931]. The crude acid was recrystallised twice from acetone at 37°, when it separated in large colourless rhombic plates, M.P. 98.3–98.5°. It gave an excellent X-ray photograph (Series B 270) in the B or long form measuring 80.4 Å., with no trace of the C or short form.

Oxidation of cocceryl alcohol to the corresponding ketonic acid. 13 g. of cocceryl alcohol dissolved in 3 litres of glacial acetic acid were treated with 7.2 g. of (moist) chromium trioxide at 65–70° for 20 minutes. The resulting green solution was poured with stirring into 7 litres of water and the solid which separated washed thoroughly with water. The salmon-pink powder thus obtained was dissolved in 2 litres of boiling 95 % alcohol, which was filtered hot. To the hot filtrate a further 200 ml. of boiling alcohol containing 10 g. of potassium hydroxide, followed by 300 ml. of boiling alcohol containing 15 g. of calcium chloride, were added. After boiling under reflux for 1 hour the mixture was filtered hot to separate the precipitated calcium soaps. These were extracted three times with 1500 ml. of boiling alcohol to remove any unchanged cocceryl alcohol and then dissolved in 600 ml. of boiling glacial acetic acid to decompose the soaps. The free ketonic acid was precipitated by pouring into excess of water, collected and washed repeatedly with water and cold acetone. It was then crystallised from 1500 ml. of acetone. The yield was 7.5 g. After recrystallisation from acetone at 37° the M.P. was 106.5–107°. (Found: C, 78.1; H, 12.9 %. $C_{34}H_{66}O_2$ requires: C, 78.1; H, 12.7 %.)

Oxime of the ketonic acid. 7 g. of the keto-acid, 7 g. of hydroxylamine hydrochloride and 12 g. of potassium hydroxide in 1500 ml. of 95 % alcohol were boiled under reflux overnight. The cooled solution was poured into 4500 ml. of dilute sulphuric acid. The solid which separated was extracted with 1200 ml. of cold ether, which was afterwards filtered. The ether was removed, and the solid residue crystallised from ice-cold acetone; 5.0 g. of oxime in microscopic needles, M.P. 62.5–63°, were obtained. (Found: C, 76.0; H, 12.6; N, 2.5 %. $C_{34}H_{66}O_2 \cdot NOH$ requires: C, 75.9; H, 12.7; N, 2.6 %.)

Transformation of the oxime and hydrolysis of the resulting amides. The procedure followed closely that observed with the oxime of 13-keto-*n*-dotriacontanoic acid. 4.5 g. of oxime gave 2.5 g. of mixed amides which were hydrolysed for 4 hours with concentrated hydrochloric acid in a sealed tube. The appropriate treatment gave ether-alcohol-insoluble material weighing 0.67 g. (Fraction A); a very small amount of benzene-alcohol-insoluble material (Fraction B) which was neglected, alcohol-insoluble soaps (Fraction C) and alcohol-soluble soaps (Fraction D).

Fraction A. This was extracted twice with boiling benzene. On cooling, the benzene deposited 0.28 g. of crystalline material. This was recrystallised from benzene, extracted thoroughly with ether and the residue crystallised from a mixture of 20 ml. of ether and 10 ml. of alcohol. 0.11 g. of *n*-nonadecanamine hydrochloride were obtained. (Found: C, 71.4; H, 13.3; N, 4.2 %. $C_{19}H_{41}N \cdot HCl$ requires: C, 71.3; H, 13.1; N, 4.4 %.) The benzoate, alone and mixed with the synthetic product, melted at 88°.

The material originally insoluble in benzene melted at 157–159°. After recrystallisation first from water and then from 2*N* hydrochloric acid it melted at 166°. The crystalline form and solubilities were similar to those of the

12-aminolauric acid hydrochloride mentioned above, and there was no doubt that it was the expected, but unknown, 14-aminomyristic acid hydrochloride. Again we found the amino-acid very difficult to purify, and after repeated treatment with boiling acetone and boiling benzene there remained only 18 mg. of material melting at 168°. (Found: (micro-Kjeldahl) N, 4.7 %. $C_{14}H_{31}N \cdot HCl$ requires: N, 5.0 %.) 13 mg. were dissolved in hot water and the requisite amount of alkali added. The free acid crystallised on cooling in thin leaflets, m.p. 181–182°. There was insufficient material for further treatment or analysis. In a mixed melt with 12-aminolauric acid (m.p. 183°) the m.p. was depressed to 168–169°.

Fraction C. The soaps were dissolved in acetic acid, which was then diluted with water. The free acids were collected and crystallised from aqueous acetone. The material thus obtained was extracted with light petroleum (b.p. < 40°). The extract on concentration yielded 38 mg. of fatty acid, m.p. 68°. This and the insoluble residue were joined with the similar products isolated from Fraction D.

Fraction D. This was treated in a similar way to Fraction C. The material soluble in cold petroleum (60 mg.), with the added material mentioned above, was recrystallised from alcohol, acetone and finally from light petroleum. It proved to be *n*-eicosanoic acid. (Found: C, 77.2; H, 12.8 %; mol. wt. by titration 308. $C_{20}H_{40}O_2$ requires: C, 76.9; H, 12.9 %; mol. wt. 312.) It melted at 74.0–74.2°, and in a mixed melt with the synthetic acid (m.p. 75–75.2°) the m.p. was 74.6–74.8°.

The residue insoluble in cold petroleum was extracted with boiling water, which was filtered hot. On cooling, a few mg. of a white crystalline solid separated, m.p. 111–112°. It was thought that this acid would be *n*-tridecane-(1:13)-dicarboxylic acid, m.p. 115°, which has been recently synthesised by Chuit [1926]. As purification of the brassylic acid in the preceding case had proved exceptionally troublesome, and as none of the synthetic acid was available for a mixed melt, the small amount of material was boiled under reflux in 5 ml. of methyl alcoholic hydrogen chloride to prepare the dimethyl ester. This melted at 42°, alone and mixed with the synthetic ester (m.p. 43°) kindly supplied by Dr Chuit. There was insufficient material for analysis.

*15-Hydroxy-*n*-tetratriacontanol.* Cocceryl alcohol (1 g.) in boiling amyl alcohol (100 ml.) was reduced with sodium (8 g. in small portions). The product was washed with water and recrystallised twice from acetone (350 ml.) at 37°, when it was obtained in small colourless rhombic plates, m.p. 98.9–99.1°. The yield was 0.7 g. (Found: C, 80.1; H, 13.8 %. $C_{34}H_{70}O_2$ requires: C, 79.9; H, 13.8 %.) The diacetate was prepared with fused sodium acetate and acetic anhydride in the usual way and recrystallised twice from a small volume of acetone at 0°, when it separated in small rosettes of colourless needles. The melting-point was not sharp, the material became molten at 49° and the melt cleared at 50–51°. (Found: C, 76.6; H, 12.4 %. $C_{38}H_{74}O_4$ requires: C, 76.8; H, 12.5 %.) It is fairly readily soluble at room temperature in ether and acetone.

SUMMARY.

Coccerin, the benzene-soluble, alcohol-insoluble wax of the cochineal insect (*Coccus cacti*) has been investigated in some detail.

The cocceryl alcohol of Liebermann is not a dihydric alcohol containing 30 carbon atoms as he suggested but is 15-keto-*n*-tetratriacontanol. His coccerinic acid is a mixture consisting of 13-keto-*n*-dotriacontanoic acid and *n*-triacontanoic acid.

The generic relationship between the ketonic acid and ketonic primary alcohol present in this wax with the secondary alcohol *n*-nonacosan-10-ol present in the wax of apple cuticle is briefly discussed.

Reasons are given for the suggestion that all previous work on the constitution of insect waxes is in need of reinvestigation.

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