XXXIII. THE FAT OF YEAST.

By ALLEN NEVILLE.

Department of Agriculture, University of Cambridge.

(Received April 24, 1913.)

Probably owing to the small quantity of material usually available, only one previous examination of yeast fat, that by Hinsberg and Roos [1903], appears to have been carried out, and, as this investigation was inconclusive on several points, it appeared to be worth while to go over the ground again. In an examination of yeast products for the "vitamines" or "hormones" connected with the phenomenon of animal growth, fairly large quantities of the crude fat of yeast became available and on this the following investigation was carried out. The results confirm the statement of the above mentioned workers that the principal saturated fatty acid present is a pentadecic acid of empirical formula $C_{15}H_{30}O_2$, but in addition the author separated a small quantity of arachidic acid, $C_{20}H_{40}O_2$. There might be noted here the diversity of opinion in the literature as to the existence of a pentadecic acid in the natural fats and the widely different values given to the physical constants of this acid by those authors who claim to have isolated it. The values given for the melting point vary from 70° to 52° , while the same constant for the methyl ester varies from 68° to 38° . Probably some of the acids described were impure as it is unlikely that a sufficient number of branched chain acids occur in the natural fats to account for the varying values given. The highest melting point obtained by the author for this acid was 59° while Hinsberg and Roos give 57° . An important point to be noticed is that for the synthetic normal acid of this composition, synthesised by Krafft [1879] and also by Le Sueur [1905], the melting point given is in both cases lower than the one found for the yeast acid, being stated as 51° by the former and 53° by the latter worker. The melting points of the simpler derivatives also disagree with those of the corresponding derivatives of the synthetic acid. The work was started in the belief that the substance was probably a mixture, but all attempts to separate it into more than one substance were unavailing and the only

conclusion possible was that the substance was really a single acid of the composition $C_{15}H_{30}O_2$ and, at the same time, that it was not the normal pentadecic acid.

In dealing with the unsaturated acids the author experienced considerable difficulty in directly isolating any pure product, the simpler derivatives being difficult to deal with and indicating no satisfactory means of separation. By oxidation with potassium permanganate, however, very satisfactory specimens of the corresponding di- and tetra-hydroxy acids were obtained and these showed the presence in the original fat of the acids $C_{16}H_{30}O_2$, $C_{18}H_{34}O_2$ and $C_{18}H_{32}O_2$. Hinsberg and Roos obtained results which they interpreted as showing the presence of the acids $C_{12}H_{22}O_2$ and $C_{18}H_{34}O_2$ though they themselves query these formulae.

The phytosterol obtained melted at $145-147^\circ$ and was apparently identical with that obtained by the other workers from one sample of beer yeast. The compound melting at 159° obtained by them from the majority of yeast samples appeared not to be present in the sample under examination.

PRELIMINARY EXAMINATION.

The crude fat was hydrolysed with alcoholic potash and after the alcohol had been distilled off the crude dry soap was extracted with ether. From the ethereal solution the yeast cholesterol was obtained. The crude soap was then redissolved in water and after neutralisation of excess of alkali, the fatty acids were precipitated in the form of their lead salts and the usual rough separation of the salts of the saturated and unsaturated acids made by extraction with ether. The free acids were then liberated from their lead salts by hydrochloric acid and treated as described below.

THE SATURATED FATTY ACIDS.

When the dark brown solid mass, obtained from the ether-insoluble lead salts, was distilled under a pressure of 100 mm. by far the larger part passed over between 250° and 260° . This was redistilled and came over at about 215° under 15 mm. pressure. In the first distillation the process was not pressed to an extreme extent and the residue left in the flask was purified by crystallisation only. The portion which had been twice distilled was crystallised several times from methyl alcohol and then melted at $54-55^\circ$. An iodine absorption determination gave however an iodine value of 29.5, and, as this was only very slowly altered by crystallisation, the acid was dissolved in dilute potash solution and treated with 1.5 per cent. permanganate

solution so as to convert the impurities consisting of unsaturated acids into the corresponding hydroxy-acids. The solution having been treated with sulphur dioxide, the precipitated acids were filtered off and treated with a small quantity of ether. Filtration from the hydroxy-acids and crystallisation of the substance obtained from the ethereal solution gave a white crystalline body, melting at 59° and showing no iodine absorption when treated with Hübl's solution.

Pentadecoic Acid.

On analysis the substance, whose preparation has just been described, gave the following result:

0.1686 g.; 0.4611 CO_2 ; 0.1878 H_2O .
 Found 74.59 % C; 12.38 % H.
 Calc. for $\text{C}_{15}\text{H}_{30}\text{O}_2$ 74.38 % C; 12.39 % H.

This substance was evidently the same as that separated by Hinsberg and Roos and described by them as being definitely pentadecoic acid.

A determination of the molecular weight by the freezing point method showed that 0.4310 g. substance dissolved in 24.2174 g. glacial acetic acid gave a depression of the freezing point of 0.295° ; whence molecular weight = 235. Calculated for $\text{C}_{15}\text{H}_{30}\text{O}_2 = 242$. On titration with alcoholic potash 1.8344 g. acid took 14.9 c.c. N/2 KOH for neutralisation.

KOH required = 22.74 % weight of acid.
 Calc. for $\text{C}_{15}\text{H}_{30}\text{O}_2 = 23.14$ % weight of acid.

The silver salt, prepared by precipitating a neutral solution of the potassium salt with silver nitrate, was obtained as a white amorphous precipitate and gave the following result on ignition:

0.4288 g. salt; 0.1308 g. metallic silver. Molecular weight of the acid = 247.
 Calc. for $\text{C}_{15}\text{H}_{30}\text{O}_2 = 242$.

The above estimations point to the substance being pentadecoic acid or a mixture with practically the same composition. If not a single compound the substance was most likely to be a mixture of two of the three acids, myristic, palmitic and stearic acid. Referring to the determinations by Heintz of the melting points of mixtures of any two of these acids it was found that with a melting point of 59° the substance might be a mixture of (a) 15 % myristic with 85 % palmitic acid, (b) 40 % myristic with 60 % stearic acid, (c) 45 % palmitic with 55 % stearic acid. Assuming the substance to be one of these mixtures, weighed quantities of pure myristic, palmitic or stearic acids were added and the melting point curves plotted. In no case did the curve agree with that for any pair of the above three acids. The melting point for a

mixture of all three of the above acids lies below that actually determined. Other methods for the separation of a possible mixture in the substance were tried but without result. Thus the fractional precipitation of the magnesium salts and the continual crystallisation of the lithium salts from alcohol were without effect. To obtain further evidence of the homogeneous nature of the substance several derivatives were prepared and compared with the corresponding compounds of the better known fatty acids.

The *methyl ester*, prepared by passing gaseous hydrochloric acid into a mixture of the acid and methyl alcohol, was obtained as a white crystalline substance which could be crystallised from a small quantity of methyl alcohol and melted at 26° , which is considerably lower than the melting point for either methyl palmitate or methyl stearate.

The *anilide* was prepared by boiling the acid for some hours with excess of aniline. It crystallised from alcohol in pearly scales and melted sharply at $86-87^{\circ}$.

Myristic anilide melts at 84° , palmitic anilide at 90.5° and stearic anilide at 94° .

Analysis: 0.2117 g.; 10.3 cc. moist N at 16° and 772 mm. $N=4.48\%$.
Calc. for $C_{21}H_{35}ON$ $N=4.41\%$.

The *amide* was prepared by heating together equal quantities of phosphorus pentachloride and the acid in chloroform. The solvent and phosphorus oxychloride were distilled off under slightly reduced pressure and the residue poured into strong ammonia. It crystallised in small flakes from 50% alcohol and melted at $94-95^{\circ}$.

The amide of the synthetic pentadecic acid, prepared by Le Sueur, melted at 102.5° , while palmitic amide melts at 107° .

0.1443 g.; 7.0 cc. moist N at 15° and 760 mm. $N=5.71\%$.
Calc. for $C_{16}H_{31}ON$ $N=5.81\%$.

The whole of the evidence points to the substance being a definite compound of the composition $C_{15}H_{30}O_2$, but at the same time being not identical with the normal synthetic acid of this composition.

Arachidic Acid.

It has been mentioned that a small quantity of material remained behind in the flask when the pentadecic acid was distilled under reduced pressure. This small quantity of material was several times crystallised from alcohol and was finally obtained as a white crystalline body which melted sharply at 77° .

On analysis it gave the following results:

0.1212 g. ; 0.3440 g. CO_2 ; 0.1385 g. H_2O .
 $\text{C} = 77.40\%$, $\text{H} = 12.70\%$.
 Calc. for $\text{C}_{20}\text{H}_{40}\text{O}_2$ $\text{C} = 76.92\%$, $\text{H} = 12.82\%$.
 0.4585 g. required 14.2 cc. $\text{N}/10$ KOH for neutralisation.
 KOH required = 17.34% of weight of acid.
 Calc. for $\text{C}_{20}\text{H}_{40}\text{O}_2$ 17.95% of weight of acid.

The substance was optically inactive, did not absorb iodine from Hübl's solution and did not react with acetic anhydride. The substance was almost certainly therefore arachidic acid.

THE UNSATURATED ACIDS.

The unsaturated fatty acids, liberated from the ether-soluble lead salts, formed a dark brown oily mass which behaved as a "semi-drying" oil when exposed in films to the air. Attempts were at once made to purify this mass by distillation under reduced pressure but without much success, as decomposition of some portion took place and the distillation was rendered useless. Distillation in steam was also of little avail the quantity passing over being very small. In an attempt to obtain some separation by means of salts, it was noticed that the barium salts, a sticky mass containing a small quantity of water, were soluble to some extent in benzene, toluene and ether, but almost insoluble in cold alcohol while with hot alcohol the mass yielded an apparently homogeneous glue with the solvent. These observations together with an iodine value of 138 for that portion of the acids whose barium salt was soluble in benzene, point to the presence in the mass of some acids of the linoleic series and the presence of some proportion of these acids would account for the "semi-drying" properties of the oil.

Attempts at direct separation of the mixture having failed, the whole "unsaturated" fraction was neutralised with caustic potash, dissolved in a large volume of water and an equal volume of 1.5 per cent. potassium permanganate solution run into it. After standing for ten minutes sulphurous acid solution was added and the precipitated acids filtered off. The filtrate was found not to contain any appreciable quantity of hydroxy-acids, thus showing the absence in the original mixture of any appreciable quantity of acids of the linoleic series.

The acids precipitated by sulphur dioxide were washed with small quantities of ether to remove unoxidised material and afterwards treated with large quantities of the same solvent. The ether-soluble portion was crystallised from alcohol and the purified dihydroxy-acid is described below. The portion insoluble in ether was crystallised from large quantities of water and was identified in the manner hereafter described as a tetrahydroxy-acid.

The substance is a white crystalline body of silky lustre, melting at 156° , soluble in hot alcohol and glacial acetic acid but insoluble in nearly all other solvents. The general properties of the acid, together with the analyses given above, point to the substance being a tetrahydroxy-acid of the composition $C_{17}H_{31}(OH)_4 \cdot COOH$. The best known acid of this composition is tetrahydroxystearic acid (sativic acid), formed by the oxidation of linoleic acid, but this melts at $173-174^{\circ}$. Apparently, however, there are several isomeric acids of this composition, for other observers have reported acids melting at 152° and 165° and the acid separated may be identical with one of these. At any rate, the isolation of this substance points definitely to the existence in yeast fat of an unsaturated acid of the linoleic series with the empirical composition $C_{18}H_{32}O_2$, that is, an acid certainly isomeric, but perhaps not identical with ordinary linoleic acid.

YEAST CHOLESTEROL.

The ethereal extract of the crude potassium salts obtained from the first hydrolysis of the yeast fat was worked up in the usual way for the yeast cholesterol. The product obtained, after being crystallised several times from alcohol, melted at $145-147^{\circ}$, and this melting point was not altered by repeated crystallisation. It had $[\alpha]_D = -75.54^{\circ}$ and gave similar colour reactions to the majority of the phytosterols. It was apparently identical with the cholesterol obtained from one sample of yeast by Hinsberg and Roos. The cholesterol, melting at 159° , which these authors most frequently obtained from yeast was not isolated in spite of a careful search being made for it.

SUMMARY.

A comparison of the results obtained by Hinsberg and Roos and by the author is shown in columns I and II respectively of the following table.

I	II
Saturated acid $C_{16}H_{30}O_2$. M.P. 56° .	Saturated acid $C_{16}H_{30}O_2$. M.P. 59° .
Not identified in the mixture.	Saturated acid $C_{20}H_{40}O_2$. M.P. 77° . Arachidic Acid.
Unsaturated acid $C_{12}H_{22}O_2$.	Not identified in the mixture.
Unsaturated acid $C_{18}H_{34}O_2$.	Unsaturated acids $C_{16}H_{30}O_4$ and $C_{18}H_{34}O_2$. Presence shown by their oxidation products.
Not identified in the mixture.	Unsaturated acid $C_{18}H_{32}O_2$, shown by its oxidation product.
Yeast cholesterol melting at 159 and $145-148^{\circ}$.	Yeast cholesterol melting at $145-147^{\circ}$.

The thanks of the author are due to Prof. T. B. Wood and Dr F. G. Hopkins, F.R.S., for kindly placing the material at his disposal and for the interest they have taken in the work throughout.

REFERENCES.

- Fahrion (1893), *J. Soc. Chem. Ind.* 936.
Hinsberg and Roos (1903), *Zeit. physiol. Chem.* 38, 1.
Krafft (1879), *Ber.* 12, 1671.
Le Sueur (1905), *J. Chem. Soc.* 87, 1898.
Ljubarsky (1898), *J. pr. Chem.* [2] 57, 19.