87. THE LIPINS OF THE EARTHWORM

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THE majority of animal fats examined in detail have been from vertebrates. In connexion with the feeding experiments described in the preceding paper, a knowledge of the composition of the mixed fatty acids of earthworms was desirable and a large quantity of worms was collected over a considerable period and kept in cold storage until sufficient was available for fatty acid analyses. In view of the unexpectedly complex mixture of acids found, a second, larger quantity of worms was collected and a second analysis carried out, which confirmed in every respect the first conclusions.

EXPERIMENTAL

In the first experiment, 25,700 g. of earthworms gave 310 g. (1.2 %) of ether-soluble matter, which was separated into 175 g. (56.4 %) of acetone-soluble "fat" (fraction A) and 135 g. of acetone-insoluble "phosphatides" (B). In the second experiment 54,450 g. of worms gave 697 g. of ether-extract (1.3 %) which gave 467 g. (67 %) of acetone-soluble "fat" (A_1) and 230 g. of "phosphatides" (B_1).

All these fractions were very dark in colour and all contained material other than fat. Fraction A gave 75.5 g. acids and 90.0 g. of unsaponifiable matter. The loss in weight largely represents water-soluble material, of unknown nature, which often accompanies crude fat extracts. Fraction A_1 gave 223 g. acids and 170 g. of unsaponifiable matter. The unsaponifiable material was mainly of a sterol-like nature [cf. Bock & Wetter, 1938] and is unusual in containing about 23 % of ergosterol. Along with these sterols there was a viscous liquid fraction. It proved unusually difficult to extract the unsaponifiable matter completely and even after 25 extractions of the aqueous soap solution with large volumes of ether several g. of unsaponifiable matter remained. This proved partly volatile during the subsequent vacuum distillation of the methyl esters, and many fractions had to be freed from traces of it before reliable equivalents could be obtained.

The acids were purified by means of light petroleum, which removed a few g. of insoluble acids. The petrol-soluble acids were subjected to lead salt-alcohol separation and the fractions esterified (by the usual process with methyl alcohol and sulphuric acid). Esterification, particularly of the solid acids, proved very incomplete, and the unesterified material, a black, sticky mass, is of unknown nature and probably polybasic. That recovered from the solid acids (of A_1) had i.v. 44.5 and sap. equiv. 240.2, whilst that from the liquid acids (A_1) had i.v. 92.0 and sap. equiv. 617.6. In calculating the final composition of the acids, the proportions of esters, rather than acids, finally resulting from the lead salt separation was used, since the non-esterifiable acids cannot be of ordinary fatty nature and it was desired to exclude them from the final results.

The "phosphatide" fractions were similarly treated but proved to contain very large proportions of non-fatty material. Fraction B gave 40 g. of petrol-soluble acids, 28 g. of petrol-insoluble acids and 11.5 g. of unsaponifiable matter. There was a large loss of water-soluble matter. Fraction B₁ gave 31.5 g. of petrol-soluble acids, 9 g. of petrol-insoluble acids and 69 g. of unsaponifiable matter. There are obviously quantitative differences between these two crude fractions. The unsaponifiable matter from B was a gummy solid, whilst that from B₁ was a liquid, both of unknown nature. The petrol-insoluble acids (from B₁) had i.v. 65.24 and sap. equiv. 288.4. Once again there was a considerable content of non-esterifiable acids. The acids from B₁, in view of the small amounts of esters finally resulting, were not submitted to fractional distillation, as the results could not have been so accurate as those already obtained for B.

Table 1. *Compositions of fatty acids of earthworm lipins (weights %)*

Fraction	Saturated acids					
	C ₁₀	C ₁₂	C ₁₄	C ₁₆	C ₁₈	C _{>18}
A	—	6	6	8	11	1
A ₁	1	4	6	6	13	2
B	—	3	2	5	8	3

Fraction	Unsaturated acids						
	C ₁₂	C ₁₄	C ₁₆	C ₁₈	C ₂₀	C ₂₂	C _{>22}
A	2 (-2.0 H)	7 (-2.7 H)	Nil ?	20 (-3.9 H)	21 (-6.8 H)	10 (- ? H)	8 (- ? H)
A ₁	3 (-2.0 H)	4 (-2.9 H)	3 (-2.8 H)	22 (-4.2 H)	26 (-6.5 H)	2 (- ? H)	8 (- ? H)
B	1	1	Nil ?	4 (-3.7 H)	42 (-4.9 H)	17 (-4.4 H)	14 (- ? H)

The lead salt separation was unusually inefficient in all cases, much higher saturated acid (e.g. stearic) going into the liquid acid fraction whilst, at the same time, much lower polyethylenic acid (e.g. C₁₄) went into the solid acid fraction. The reason for this is quite obscure. It was necessary to oxidize a great many of the fractions by the permanganate-acetone method [Harper *et al.* 1937] before their compositions could be calculated.

The compositions of the esterifiable fatty acid mixtures are given in Table 1. In view of the unusual complexity of these lipins and the difficulties encountered during the analysis (e.g. the presence of volatile unsaponifiable matter), these results cannot be regarded as of the usual order of accuracy. In addition to the esterifiable acids, the crude acid mixtures contained 17, 10 and 54 % of "non-esterifiable" (and petrol-insoluble) acids for A, A₁ and B respectively. B₁ acids contained 72 % of such non-fatty acids.

DISCUSSION

It will be seen from Table 1 that the two analyses for the acetone-soluble fraction are in good agreement considering the difficulties mentioned. The phosphatide acids are in general of a similar nature, but contain rather less of saturated acids and much less C₁₈ unsaturated, whilst the contents of higher unsaturated acids are much larger. In each case the saturated acids with more than 18 carbon atoms had a mean equiv. wt. of about 350, and were probably a mixture. The unsaturated acids with more than 22 carbon atoms were also presumably a mixture and had mean equiv. wt. of over 400.

Points of interest in these unusual acid mixtures include the presence of lower mol. wt. derivatives, both saturated and unsaturated, the very low content of hexadecenoic acid, the high ratio of stearic to palmitic acid and the high degree of average unsaturation of the C_{14} and C_{18} acid groups. It did not prove possible to obtain reliable figures for the average unsaturation of each group of C_{22} and $C_{>22}$ acids, but all were certainly polyethylenic.

In view of the high content of unsaponifiable matter in the acetone-soluble fractions it seems doubtful whether much ordinary triglyceride could have been present, most of the fat presumably being sterol, etc., esters.

The unusual nature of these fatty acid mixtures indicates the desirability of obtaining data for other classes of invertebrates which have not so far been examined.

SUMMARY

The acids occurring in the acetone-soluble and acetone-insoluble fractions respectively of earthworm lipins have been examined. In addition to acids of unknown (possibly non-fatty) nature, these contain fatty acids ranging from C_{10} to $C_{>22}$, including unsaturated C_{12} acids. Ordinary triglyceride cannot be an important constituent in view of the high content of sterols.

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