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FATTY ACID COMPOSITION OF THE MILLIPEDE *GRAPHIDOSTREPTUS TUMULIPORUS* (Karsch) (MYRIAPODA: DIPLOPODA)

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Abstract—1. The fatty acid composition of female specimens of the millipede *Graphidostreptus tumuliporus* is investigated.

2. This composition differs remarkably from the composition of the male specimens of *Graphidostreptus*, as given in an earlier paper.

3. Over 60 per cent of the total fatty acid composition in the study is satura ted, nearly half of it of an unusual branched type.

4. The content of polyunsaturated fatty acids is rather low, the most abundant unsaturated component is 18:1 (18·10 per cent).

INTRODUCTION

IN THE class Myriapoda of the phylum Arthropoda, data concerning the composition and metabolism of lipids are fragmentary, as are other aspects of metabolism. Only the defensive secretions of millipedes have been studied in more detail (Schildknecht & Weiss, 1961; Schildknecht & Wenneis, 1966; Schildknecht *et al.*, 1967; Weatherston, 1967; Eisner & Meinwald, 1966). Shrivastava (1970) studied cuticular components of Indian species. During investigations into the composition and biosynthesis of hydrocarbons in the millipede *Graphidostreptus tumuliporus* (Karsch) (Oudejans, to be published) it became evident that the composition of the fatty acids should be known, because it has been argued that there might be a relationship between the biosythesis of fatty acids and hydrocarbons. (For reviews see: Albro (1970), Kolattukudy (1970a, b)).

Zandee (1964, 1967) has already published a list of the fatty acids of male specimens of *G. tumuliporus*, and also data concerning their biosynthesis after injection with 1-^{14}C-acetate. This study made it clear that the composition of the fatty acids in this millipede is more complicated than that of most of the insects (Fast, 1964); this organism is therefore more related to the other classes of the phylum Arthropoda with a more complex fatty acid composition, especially the Crustacea and Arachnida (for instance Barlow, 1964; Zandee, 1964, 1966, 1967; Collatz, 1969; Ackman & Hooper, 1970).

In the present paper the composition of the fatty acids of *G. tumuliporus* is reinvestigated in female animals, determined with more advanced techniques of

extracting and separating lipids and by means of gas chromatography using the highly sensitive flame ionization detector instead of the katharometer detector formerly used.

MATERIALS AND METHODS

Nineteen ♀♀ of an African millipede, *Graphidostreptus tumuliporus* (Karsch) were received from the "Institut Fondamental d'Afrique Noire", Dakar, Sénégal. The animals had been collected in the vicinity of the institute.

After homogenizing the animals in a mixer, lipids were extracted three times with chloroform–methanol 2 : 1 (v/v). The extract was washed with 0·2 vol. of a 0·73% NaCl solution, evaporated to dryness with a vacuum rotator and saponified by refluxing in a solution of 1·5 N KOH in 80% methanol for 6 hr at 60–70°C in an atmosphere of nitrogen.

Non-saponifiable lipids were extracted with redistilled hexane (p.a.). The residue was acidified with concentrated HCl to pH 1, after which the fatty acids were extracted with redistilled hexane (p.a.). Part of the fatty acids was methylated with diazomethane (Schlenk & Gellerman, 1960) and the methylesters were separated from pigments on a silicic acid column (Mallinckrodt, 100 mesh) (Allen, 1968). Samples of the total methylated fatty acids were also hydrogenated according to Farquhar *et al.* (1959).

Gas chromatography of the total and hydrogenated methylated fatty acids was performed on a Becker instrument, Model 1452, equipped with dual flame ionization detection. Two columes were used:

1. Stainless steel, 1·80 m × 3·8 mm i.d., packed with 20% PEGA on acid- and alkaline-washed Chromosorb W (60–70 mesh); the column temperature was 170°C whilst the flow of carrier gas (N_2) was 50 ml/min.

2. Glass, 1·80 m × 3·8 mm i.d., packed with 10% Apiezon L. on acid- washed Chromosorb W (100–120 mesh); the column temperature was 170°C whilst the flow of carrier gas (N_2) was 80 ml/min.

The qualitative and quantitative composition was calculated as described by van der Horst (1970).

RESULTS AND DISCUSSION

The quantities of the isolated lipid fractions are summarized in Table 1.

TABLE 1—QUANTITIES OF ISOLATED LIPID FRACTIONS

	Weight (g)	Fresh weight (%)
Fresh weight	251·90	100
Total lipids	16·18	6·42
Non-saponifiable lipids	1·5759	0·62
Saponifiable fraction	12·1128	4·80
Fatty acids as methylester	11·5072	4·56

The quantities of these fractions are larger than given by Zandee (1967) because the animals were not saponified *in toto*, but the lipids were extracted before saponification. Moreover, the saponifiable fraction was not decolorized with acid-washed activated charcoal, which may give selective adsorption of certain fatty acids. The unsaponifiable lipids will be discussed in our next paper.

The composition of the fatty acids is summarized in Table 2 whilst in Table 3 the total percentages of different homologous series of fatty acids are given.

TABLE 2—COMPOSITION OF THE FATTY ACIDS (in mol %), CALCULATED FROM CHROMATOGRAMS OF TOTAL AND HYDROGENATED SAMPLES ON TWO DIFFERENT STATIONARY PHASES

Name	Total	Hydrogenated	Name	Total	Hydrogenated
< 14:0 br	1·60	1·71	19:1	1·16	—
14:0 br	0·03	0·04	C*	10·00	10·00
14:0	0·24	0·31	20:0 iso	0·34	0·17
14:2	0·06	—	20:0	1·43	4·15
15:0 iso	0·12	0·14	20:1	2·08	—
15:0 br	0·03	0·03	20:2	0·38	—
15:0	0·80	0·99	20:4	0·25	—
15:1	0·21	—	21:0 br	0·08	0·08
16:0 iso	0·18	0.15	21:0	0·20	0·27
16:0 br	0·04	0·04	21:1	0·12	—
16:0	16·25	19·00	22:0 br	0·17	0·13
16:1	2·50	—	22:0	0·46	0·81
16:2	0·02	—	22:1	0·40	—
17:0 iso	0·32	0·30	23:0 br	0·14	0·12
17:0	1·68	4·02	23:0	0·14	0·25
17:1	2·01	—	23:1	0·23	—
A*	1·74	1·74	24:0 br	0·19	0·18
18:0 iso	0·10	0·10	24:0	0·33	0·33
18:0 br	0·28	0·27	25:0 br	0·27	0·27
18:0	4·60	33·15	25:0	0·10	0·07
18:1	18·10	—	26:0 br	0·15	0·15
18:2	8·05	—	26:0	0·08	0·08
18:3	2·48	—	27:0 br	0·15	0·15
B*	18·00	18·00	27:0	0·09	0·09
19:0 iso	trace	trace	28:0 br	0·11	0·11
19:0 br	0·31	0·35	28:0	0·18	0·18
19:0	0·48	1·57			

* A, B and C: unknown saturated fatty acids discussed in the text.

TABLE 3—TOTAL PERCENTAGES OF THE DIFFERENT HOMOLOGOUS SERIES OF FATTY ACIDS

Series	Total	Hydrogenated
Branched ("neo")	1·95	1·92
Iso-branched	1·06	0·86
A, B and C	29·74	29·74
Saturated straight-chain	27·06	65·27
Mono-unsaturated	26·81	—
Di-unsaturated	8·51	—
Tri-unsaturated	2·48	—
Tetra-unsaturated	0·25	—

The most abundant components appear to be palmitic acid (16:0) and oleic acid (18:1) and also two of the three unknown saturated fatty acids, viz. B and C. An outstanding fact is the very low amount of polyunsaturated fatty acids as compared with some molluscs investigated in our laboratory (van der Horst & Voogt, 1969a, b; van der Horst, 1970).

In the lipids of *Graphidostreptus* some different homologous series of the branched-chain saturated fatty acids were found.

Iso-branched series

Of the total fatty acids, 1·06 per cent, the most important components are 20:0 iso and 17:0 iso.

A–B–C series

These three components showed a behaviour on the Apiezon column similar to an anteiso-branched series of standard fatty acids (Applied Science Labs.) (compare the relative retention times in Table 4), but surprisingly, on the PEGA-column they behaved like a series with shorter elution times than the iso-branched series.

TABLE 4—RELATIVE RETENTION TIMES (18:0 = 1·0000) OF COMPONENTS A, B AND C ON TWO DIFFERENT STATIONARY PHASES COMPARED WITH SOME STANDARD FATTY ACIDS

	PEGA		Apiezon	
	Total	Hydrogenated	Total	Hydrogenated
A	(0·7829)*	0·7562	0·5424	0·5465
B	(1·0881)†	1·0660	0·8759	0·8693
C	(1·5445)‡	1·5130	1·3880	1·3830
17:0 ante-iso		0·6382		0·5460
18:0 ante-iso		0·9020		0·8668
19:0 ante-iso		1·2840		1·3760

* Complex peak together with 17:1. † Complex peak together with 18:1. ‡ Complex peak together with 19:1.

Moreover, on the Apiezon column A, B and C seemed to be fatty acids with 17, 18 and 19 C-atoms respectively, whilst on the PEGA-column they had 18, 19 and 20 C-atoms respectively, so that the exact chain length of the components is yet unknown. These unknown fatty acids are saturated ones, because their retention times do not change upon hydrogenation.

The different identification on both columns somewhat resembles that of hydroxy fatty acids, for example 14:0 hydroxy behaves on an Apiezon column like a 18:0 branched-chain fatty acid, and on a PEGA-column like arachidic acid (20:0). However, standard hydroxy fatty acids show on the Apiezon column a strong tailing and this does not apply to components A, B and C (Figs. 1, 2).

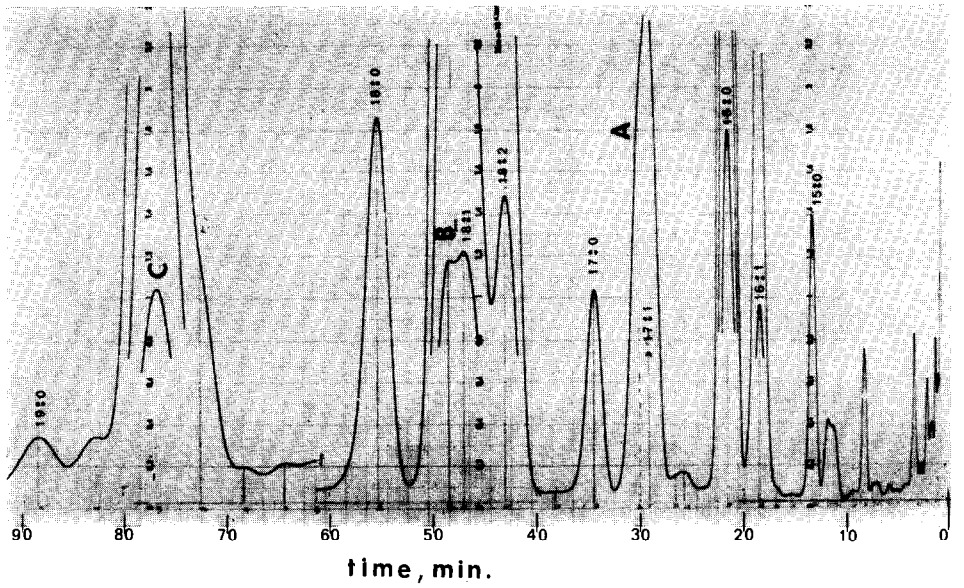

FIG. 1. Part of the gas chromatogram of the total methylated fatty acids of *G. tumuliporus* (females) on an Apiezon column.

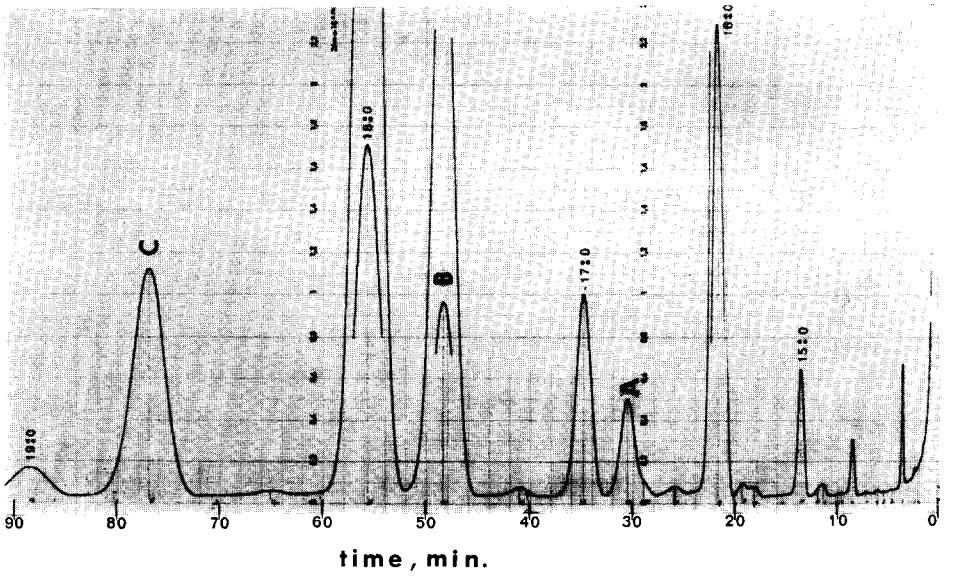

FIG. 2. Idem, hydrogenated sample.

Our tentative identification of the structure is that it is a multiple-branched one. This may be supported by the fact that also in the hydrocarbons there are probably multiple-branched ones. The long-chain fatty acids also point to a relationship between the biosynthesis of fatty acids and hydrocarbons: the first can be considered as being by-products of the biosynthesis of the hydrocarbons (Kolattukudy, 1970b).

Branched series

The components of this series behave on both columns as the neo-branched series tentatively identified by Zandee (1967). The A, B and C fatty acids on the PEGA column also remarkably behaved as neo-branched ones, but on the Apiezon column their elution times differ from those of the other members of the tentatively identified neo-branched series. This so-called neo-type of branching could be an internally branched type as well (Ackman, 1967).

The most important components of the straight-chain saturated fatty acids are: 16:0 (16·25 per cent) and 18:0 (4·60 per cent), whilst in the unsaturated ones the most abundant acids are 18:1 (18·10 per cent), 18:2 (8·05 per cent), 16:1 (2·50 per cent), 18:3 (2·48 per cent) and 20:1 (2·08 per cent).

The high amount of fatty acids named A, B and C (29·74 per cent of the total fatty acids) appears to be a unique feature of the female millipede, and we are therefore carrying out further investigations into the exact nature and occurrence of these components.

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Key Word Index—Fatty acids; millipedes; *Graphidostreptus tumuliporus*; female fatty acid composition in millipedes.