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OCCURRENCE OF CYCLOPROPANE FATTY ACIDS IN FEMALES AND EGGS OF THE MILLIPEDE *GRAPHIDOSTREPTUS TUMULIPORUS* (KARSCH) (MYRIAPODA: DIPLOPODA), AS CONTRASTED WITH THEIR ABSENCE IN THE MALES

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Abstract—1. The distribution of cyclopropane fatty acids in the lipids of males, females and eggs of the millipede *Graphidostreptus tumuliporus* is investigated.

2. Cyclopropane fatty acids account for 25 per cent of the total fatty acids in the female animals (without eggs), and for as much as 35 per cent in the eggs, whilst in the males the presence of these fatty acids could not be detected.

3. In contrast to nearly all bacteria, protozoa and plants cyclopropane fatty acids occur in this millipede not only in the phospholipids but also in the neutral lipids.

4. Some aspects of the structure, metabolism and function of cyclopropane fatty acids in *Graphidostreptus* are discussed.

INTRODUCTION

CYCLOPROPANE fatty acids have been reported to occur only in bacteria, plants and protozoa (review, Christie, 1970). Except for plants these cyclopropane fatty acids are exclusively constituents of the phospholipids, viz. predominantly esterified in the 2-position.

Recently we have isolated a large amount of these unusual fatty acids from female specimens of an African millipede, *Graphidostreptus tumuliporus* (Karsch). The high specific radioactivy of these components after injection of the animals with 1-^{14}C-acetate indicated that they must play an important role in the lipid metabolism (Oudejans *et al.*, 1971a, b). However, from earlier studies on the fatty acid composition of males of the same species (Zandee, 1964, 1967) no indications for the presence of cyclopropane fatty acids were obtained.

This remarkable discrepancy may be due to the fact that the female animals used in our experiments always contained large amounts of eggs.

Therefore, in this study eggs were removed and their fatty acid composition was compared with those of the males and females (without eggs).

In order to investigate the possibility that the occurrence of cyclopropane fatty acids is perhaps a common feature of millipedes, we also studied the composition of the fatty acids from another species, *Julus scandinavius* Latzel.

MATERIALS AND METHODS

Seventeen females and six males of *Graphidostreptus tumuliporus* (Karsch) (order Spirostreptida) were received in October 1970 from the Institut Fondamental d'Afrique Noire, Dakar, Senegal.

At the end of March 1971, thirty-three specimens of *Julus scandinavius* Latzel (order Julida) (sex not determined) were collected in the vicinity of Utrecht.

From the female animals of *G. tumuliporus*, eggs were removed and worked up separately.

From the different groups lipids were extracted and fatty acids isolated as described elsewhere (Oudejans *et al.*, 1971a). Samples of the fatty acids were methylated with diazomethane (Schlenk & Gellerman, 1960) and separated from pigments on a silicic acid column (Mallinckrodt, 100 mesh) with hexane–ether 95:5 (v/v).

A part of each sample was hydrogenated in absolute methanol with platinum oxide as a catalyst under positive hydrogen pressure at room temperature.

To study the distribution of cyclopropane fatty acids in different lipid classes, phospholipids of a separate group of female animals (including eggs) were isolated from the total lipids according to Lipsky *et al.* (1957).

Analytical gas chromatography was performed on a Becker 1452 instrument on two different stationary phases, viz. 10% Apiezon L and 20% PEGA under the conditions previously described (Oudejans *et al.*, 1971b). Moreover, for the analysis of very long-chain fatty acids (> 25 C-atoms), SE 52 was used as a third stationary phase. Chromosorb W (100–120 mesh) was precoated with 5% dichlorodimethylsilane in toluene and coated with 4% SE 52. Dimensions of the aluminium column used were 1·80 m × 4 mm i.d., the column temperature was 170°C, whilst the outlet flow of the carrier gas (nitrogen) was 50 ml/min.

The analyses on this column were carried out on a Becker 2300 instrument which was also equipped with a flame ionization detector.

Fatty acid compositions were calculated as described by van der Horst (1970). Besides the usual standard fatty acids the cyclopropane fatty acids, of which we have already described the isolation and identification in an earlier paper (Oudejans *et al.*, 1971b), were used as reference fatty acids.

RESULTS

Table 1 summarizes the various weights of the different groups of animals and their lipid fractions.

Table 1—Quantities of the different lipid fractions of *G. tumuliporus* and *J. scandinavius*

	G. tumuliporus			*J. scandinavius*
	Males	Females	Eggs	Males, Females
Number of animals	6	17	—	33
Fresh weight (g)	52·3	211·3	23·9	1·9687
Total lipid (g)	1·1716	4·0521	3·4527	0·0342
(% of fresh weight)	2·24	1·92	14·45	1·74
Unsaponifiable lipids (g)	0·1864	0·4117	0·1513	0·0045
(% of fresh weight)	0·36	0·20	0·63	0·23
Saponifiable lipids (g)	0·5932	3·2547	2·5011	0·0166
(% of fresh weight)	1·13	1·54	10·47	0·84
Fatty acids as methyl esters (% of saponifiable lipids)	83·9	68·4	106·2	*

* Not determined.

The compositions of the fatty acids of the total lipids are listed in Table 2, whilst the percentages of the different homologous series of fatty acids are given in Table 3.

TABLE 2—FATTY ACID COMPOSITIONS (in mol %) OF THE TOTAL LIPIDS OF TWO MILLIPEDES

Fatty acid	*G. tumuliporus*			*J. scandinavius*
	Males	Females	Eggs	Males, Females
<14:0 br*	0·62	0·39	0·10	0·39
14:0 br	0·04	0·07	0·03	0·21
14:0	0·32	0·27	0·22	0·84
14:1	—	—	—	0·18
14:2	0·10	0·05	0·08	—
15:0 iso	0·10	—	0·19	—
15:0 br	0·10	0·16	Trace	1·17
15:0	0·62	0·66	1·00	2·26
15:1	0·22	0·23	0·15	1·35
16:0 iso	0·13	—	0·40	—
16:0 br	—	0·20	0·05	1·16
16·0	18·29	15·31	12·46	10·62
16:1	4·52	2·95	1·90	4·16
16:2	Trace	—	Trace	0·67
17:0 iso	Trace	0·66	0·22	—
17:0 br	0·36	—	0·31	1·61
17:0	0·81	1·69	2·09	3·50
17:1	1·96	2·47	2·49	2·17
17:0 cyclo†	—	1·70	2·62	—
18:0 iso	0·37	—	0·14	—
18:0 br	—	—	0·09	0·76
18:0	6·34	5·21	4·90	3·56
18:1	35·94	22·95	19·51	17·71
18:2	11·58	8·32	8·86	19·88
18:3	4·25	2·56	2·72	4·84
18:0 cyclo	—	14·34	19·58	—
19:0 iso	Trace	—	0·16	—
19:0 br	—	0·24	0·08	3·85
19:0	0·91	0·29	0·50	0·22
19:1	0·49	1·40	1·58	1·59
19:0 cyclo	—	8·61	12·35	—
20:0 iso	0·82	0·28	0·22	—
20:0 br	—	—	—	1·05
20:0	3·41	2·02	1·30	2·25
20:1	4·10	3·40	1·99	1·20
20:2	0·44	0·24	0·36	0·24
20:4	0·30	0·59	0·21	4·05

* Br, branched-chain fatty acids.
† Cyclo, cyclopropane fatty acids.

Table—2 continued overleaf.

Table 2—*continued*

Fatty acid	*G. tumuliporus* Males	*G. tumuliporus* Females	*G. tumuliporus* Eggs	*J. scandinavius* Males, Females
20: ?	—	—	—	2·62
21:0 br	Trace	0·08	0·05	0·43
21:0	0·20	0·10	0·08	0·31
21:1	0·03	0·02	Trace	0·05
22:0 iso	0·04	—	0·07	—
22:0 br	0·18	0·21	—	0·34
22:0	0·46	0·35	0·20	0·11
22:1	0·19	0·07	0·17	0·44
23:0 br	0·06	0·09	0·13‡	—
23:0	0·15	0·08	0·04	0·20
23:1	0·10	0·08	Trace	0·16
24:0 br	0·07	0·44	0·02	—
24:0	0·23	0·32	0·15	0·47
25:0 br	0·17	0·16	Trace	0·23
25:0	0·10	0·05	0·02	0·34
26:0 br	0·11	Trace‡	0·02	—
26:0	0·13‡	0·09	0·03	1·17
27:0 br	0·08	Trace	—	0·58
27:0	0·05	0·17	Trace	1·02
28:0 br	0·10	0·04	—	—
28:0	0·14	0·15	0·05	0·03‡
29:0 br	0·07	0·04	—	—
29:0	0·04	0·04	Trace	Trace
30:0 br	0·05	Trace	—	—
30:0	0·07	0·10	0·03	—

‡ From this fatty acid on, the following components were only determined on the SE 52 column.

Table 3—Total percentages of the different homologous series of fatty acids

Series	*G. tumuliporus* Males	*G. tumuliporus* Females	*G. tumuliporus* Eggs	*J. scandinavius* Males, Females
Cyclopropane	—	24·65	34·55	—
Saturated straight-chain	32·27	26·90	23·07	26·90
Saturated branched-chain	2·85	2·67	2·18	11·39
Mono-unsaturated	47·55	33·57	27·79	29·01
Di-unsaturated	12·12	8·61	9·30	20·79
Tri-unsaturated	4·25	2·56	2·72	4·84
Tetra-unsaturated	0·30	0·59	0·21	4·05
?-unsaturated	—	—	—	2·62

From 1·240 g of total lipids obtained from a separate group of females (including eggs), 0·2845 g of phospholipids were isolated (22·95 per cent of the total lipids). The fatty acids (0·0963 g) of these phospholipids consisted of 21·55 per cent of cyclopropane fatty acids, but in the neutral lipids as much as 31·00 per cent of the fatty acids turned out to have the same structure.

DISCUSSION

From Table 2 it is clear that remarkable differences exist in the fatty acid compositions of the male, female and egg lipids of *G. tumuliporus*.

Females and eggs contain very high amounts of three cyclopropane fatty acids, whilst the presence of these fatty acids could not be detected in the male specimens.

The occurrence of such large amounts of cyclopropane fatty acids in only one sex of this millipede points to a rather different lipid metabolism in males and females.

The lipid quantity of the eggs is quite high (14·45 per cent of the fresh weight) in comparison to that of the adults (Table 1). The fact that the content of cyclopropane fatty acids in these egg lipids is even higher than that of the females suggests that they perform an important function in the lipid structure of the eggs.

In the phospholipids of some bacteria monoenoic fatty acids are replaced by cyclopropane derivatives, by which no appreciable changes are made in the physical properties of the phospholipids (Law, 1967; Christie, 1970). It is not known, however, why in organisms cyclopropane fatty acids should be preferred for this purpose to monoenoic acids or what advantages these components offer in spite of the energetically expensive reactions involved in their biosynthesis.

In the lipids of *Julus scandinavius* we were unable to detect cyclopropane fatty acids, so the occurrence of these acids does not seem to be a common feature of millipedes. Bacterial cyclopropane fatty acids are synthesized by the addition of the methylene group derived from the methyl group of S-adenosylmethionine across the double bond of a monoenoic acyl moiety of phosphatidyl ethanolamine (Lin & Hofmann, 1962; O'Leary, 1962; Zalkin *et al.*, 1963; Chung & Law, 1964; Kates, 1964; Thomas & Law, 1966).

Even in flagellates where phosphatidyl choline is the major phospholipid, phosphatidyl ethanolamine is the specific substrate of this synthesis (Meyer & Holz, 1966). The occurrence of cyclopropane fatty acids in nearly all bacteria and flagellates is restricted to this specific phospholipid. However, in *G. tumuliporus* this type of fatty acids was present not only in the phospholipid fraction, but also in an even higher amount in the neutral lipids.

Thus, either the biosynthesis of these acids is different from that in other organisms, or by transport or conversion these acids occur in the neutral lipids.

An interesting fact is the different number of carbon atoms of the cyclopropane fatty acids of *G. tumuliporus* (a series of 17, 18 and 19 C-atoms, respectively) as compared with the acids which have been isolated from bacteria and protozoa (all with an odd number of C-atoms) (Goldfine & Bloch, 1961; O'Leary, 1962; Meyer & Holz, 1966; Park & Berger, 1967).

In plants also, in which the occurrence of cyclopropane and cyclopropene fatty acids is restricted to the order Malvales, only odd-numbered acids have been reported (Hooper & Law, 1965; Johnson *et al.*, 1967).

Both odd- and even-numbered cyclopropane fatty acids of *G. tumuliporus* turned out to be highly radioactive after injection of the animals with 1-^{14}C-acetate (Oudejans *et al.*, 1971b).

The metabolism of these compounds and their function in egg lipids will be studied in more detail at a later date.

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Key Word Index—Cyclopropane fatty acids; millipedes; *Graphidostreptus tumuliporus*; *Julus scandinavius*; lipid metabolism male and female millipedes.

FIG. 1. Two-dimensional thin-layer chromatogram of the phospholipids of adult *S. granarius*. Solvent systems used: first dimension—chloroform–methanol–7 N ammonium hydroxide–water (65 : 35 : 5 : 2·5 v/v): second dimension—chloroform–methanol–butanol–acetic acid–water (90 : 60 : 40 : 20 : 15 v/v). Legend: O, origin; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; PS, phosphatidyl serine; LPE, lysophosphatidyl ethanolamine; LPC, lysophosphatidyl choline; Sph, sphingomyelin; PI(?), possibly phosphatidyl inositol; NL, neutral lipids; X_1 and X_2, unknowns.

TABLE 2—THE PHOSPHOLIPID COMPOSITION OF *S. Granarius* L. AT THREE ADULT AGES*

	g/100 g Phospholipid					
	GG strain			MW strain		
Phospholipid class	0–6 days	14–21 days	56–63 days	0–6 days	14–21 days	56–63 days
Phosphatidyl ethanolamine	45·5 ± 1·65	46·4 ± 0·86	46·3 ± 0·59	45·8 ± 0·67	45·8 ± 1·31	45·6 ± 1·33
Phosphatidyl choline	28·2 ± 0·99	28·4 ± 1·10	26·6 ± 0·25	29·8 ± 0·94	29·3 ± 1·07	28·2 ± 0·42
Phosphatidyl serine	9·8 ± 1·10	9·8 ± 0·88	10·5 ± 0·51	10·6 ± 0·65	9·2 ± 0·99	10·1 ± 1·05
Other phospholipids	16·5 ± 0·93	15·4 ± 1·24	16·6 ± 0·43	13·8 ± 0·47	15·7 ± 2·59	16·1 ± 0·61

* Mean and standard deviation of five separate analyses for each age.

PC was present during all three ages, these differences seem too slight to call for an explanation.

The phospholipid pattern of *S. granarius* is similar to that of higher Diptera (Bieber *et al.*, 1961; Crone & Bridges, 1963; Fast, 1966), where PE is the major phospholipid fraction. This situation is unlike that reported for most other insects (Hodgson, 1965; Wlodawer & Wisniewska, 1965; Fast, 1966, 1967; Kinsella, 1966, 1969, 1970; Lipsitz & McFarlane, 1970), where PC is the principal phospholipid fraction, and unlike that of other Coleoptera reported on where the PE and PC fractions are equal (Kamienski *et al.*, 1965; Fast, 1966; Rao & Agarwal, 1969; Henson *et al.*, 1971) or PC is the principal fraction (Willis & Hodgson, 1970). The other differences in the phospholipid patterns between *S. granarius* and the few other Coleoptera reported on are the presence of both lysophosphatides and PS (see Kamienski *et al.*, 1965; Rao & Agarwal, 1969).

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Key Word Index—Lipids; insects; *Sitophilus granarius*; Coleoptera; Curculionidae; granary weevil; phospholipids; symbiotes; mycetomes.