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COMPOSITION OF THE SATURATED HYDROCARBONS FROM MALES, FEMALES, AND EGGS OF THE MILLIPEDE, *GRAPHIDOSTREPTUS TUMULIPORUS*

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Abstract—The total hydrocarbons of the millipede *Graphidostreptus tumuliporus* contain 10 per cent saturated components. The composition of the saturated hydrocarbons from males, females, and eggs is reported.

Straight-chain alkanes (n-C_{15}–n-C_{36}) constitute 59 to 75 per cent of the saturated hydrocarbons with a strong predominance of odd chain over even chain homologues. Several types of branched-chain alkanes are also present: 2-methyl (*iso*), 3-methyl (ante*iso*), and at least one other type of branching.

In males and females the most abundant n-alkane is nonacosane (n-C_{29}) (21·67 and 32·10 per cent, respectively), whereas in eggs heptacosane (n-C_{27}) predominates (9·90 per cent). The composition of the n-alkanes in males and females resembles the values obtained for plant hydrocarbons, indicating that part of the saturated hydrocarbons is probably derived from the food. The low content of n-C_{29} in the eggs (7·70 per cent) may indicate that the hydrocarbons of the ingested food are not deposited into the egg lipid in equal amounts.

INTRODUCTION

In arthropods hydrocarbons are important constituents of cuticular lipids, although they are also found in the fat body and haemolymph.

In addition to the ubiquitous n-alkanes several types of branched-chain and unsaturated hydrocarbons have been identified (Baker *et al.*, 1960, 1963; Louloudes *et al.*, 1962; Hutchins and Martin, 1968; Cavill *et al.*, 1970; Jackson and Baker, 1970; Martin and MacConnell, 1970; Nelson and Sukkestad, 1970; Tartivita and Jackson, 1970; Nelson *et al.*, 1971).

Recently the incorporation of several precursors into the total hydrocarbon fraction of the millipede *Graphidostreptus tumuliporus* (Karsch) was reported (Oudejans, 1972). In the present paper the composition of the saturated hydrocarbons from males, females, and eggs of this species is presented.

MATERIALS AND METHODS

Twenty-five male and 17 female specimens of the millipede *Graphidostreptus tumuliporus* (Karsch) were received from the Institut Fondamental d'Afrique Noire, Dakar, Senegal, in October 1970. Eggs were removed and worked up separately.

Lipids were extracted and saponified as described elsewhere (OUDEJANS *et al.*, 1971a). The unsaponifiable fractions were chromatographed on alumina (Merck) (VOOGT, 1970, 1971) and hydrocarbons were eluted with redistilled hexane as the first eluens. Saturated hydrocarbons were separated from unsaturated components by chromatography on silica gel impregnated with 25% silver nitrate (Adsorbosil-CABN, 140 mesh, Applied Science Laboratories) with redistilled hexane. In order to detect traces of possibly unsaturated contaminants, parts of the saturated hydrocarbons were hydrogenated with platinumoxide in 2,2,4-trimethylpentane (Uvasol®, Merck) according to LOULOUDES *et al.* (1962). Gas–liquid chromatography of the hydrocarbons was carried out on 4% SE-52 and 20% polyethyleneglycoladipate (PEGA) under the conditions described in an earlier paper (VAN DER HORST and OUDEJANS, 1972).

Moreover 10% Apiezon L was used as a third stationary phase under the conditions described formerly (OUDEJANS *et al.*, 1971b).

Qualitative and quantitative compositions were calculated using the same standard hydrocarbons as mentioned by VAN DER HORST and OUDEJANS (1972).

In order to avoid contamination with other hydrocarbons, all solvents were redistilled before use and blanks were run throughout the extraction and chromatographic procedures.

RESULTS

The quantities of the isolated lipids and the hydrocarbon fractions are summarized in Table 1.

TABLE 1—QUANTITIES OF ISOLATED TOTAL LIPID FRACTIONS AND HYDROCARBONS IN MALES, FEMALES, AND EGGS OF *G. tumuliporus*

	Males	Females*	Eggs
Number	25	17	—
Fresh weight (g)	205·9	211·3	23·9
Total lipids (g)	3·6246	4·0521	3·4527
(% of fresh weight)	1·76	1·92	14·45
Unsaponifiable lipids (g)	0·2077	0·4117	0·1513
(% of total lipids)	5·73	10·16	4·38
Hydrocarbons (g)	0·0997	0·2061	0·0650
(% of unsaponifiable lipids)	48·0	50·1	42·9
Saturated hydrocarbons (g)	0·0119	0·0186	0·0060
(% of hydrocarbons)	11·9	9·0	9·2

*Eggs removed.

Hydrocarbons appear to constitute 40 to 50 per cent of the unsaponifiable lipids in all groups.

The amount of saturated hydrocarbons is surprisingly low, viz. some 10 per cent of the total hydrocarbons. Until now there have been but few organisms with

TABLE 2—COMPOSITION (IN mol. %) OF THE SATURATED HYDROCARBONS FROM MALES, FEMALES, AND EGGS OF *G. tumuliporus*

	Males	Females	Eggs
< n-C_{15}	0·10	1·15	0·61
n-C_{15}	0·18	0·15	0·39
br-C_{16}	0·06	0·03	0·13
i+a-C_{16}	0·07	0·06	0·14
n-C_{16}	0·48	0·87	0·65
br-C_{17}	0·25	0·05	0·21
i+a-C_{17}	0·15	0·05	0·13
n-C_{17}	1·20	0·42	1·15
br-C_{18}	0·49	0·09	0·19
i+a-C_{18}	0·48	0·16	0·32
n-C_{18}	1·44	0·72	1·53
br-C_{19}	0·34	0·13	0·21
i-C_{19}	0·91	0·26	0·43
a-C_{19}			0·61
n-C_{19}	1·26	0·39	1·68
br-C_{20}	0·71	0·15	0·19
i-C_{20}	0·89	0·28	0·99
a-C_{20}			0·64
n-C_{20}	1·39	0·33	1·50
br-C_{21}	0·74	0·21	0·36
i-C_{21}	0·76	0·36	0·76
a-C_{21}	0·62	0·19	0·49
n-C_{21}	1·20	0·49	1·58
br-C_{22}	0·95	0·26	0·34
i-C_{22}	Trace	—	—
a-C_{22}	0·96	0·34	0·48
n-C_{22}	1·35	0·52	5·01
br-C_{23}	2·00	0·28	0·29
i-C_{23}	1·36	0·14	0·32
a-C_{23}		0·16	—
n-C_{23}	2·25	1·59	5·23
?	1·58	—	—
br-C_{24}	1·61	0·49	0·65
a-C_{24}	0·64	Trace	0·23
n-C_{24}	1·65	3·55	2·45
br-C_{25}	1·14	0·47	0·81
i-C_{25}	0·76	0·41	0·66
n-C_{25}	2·91	3·75	5·95
br-C_{26}	0·99	0·93	0·77
a-C_{26}	0·75	0·41	0·50
n-C_{26}	1·98	1·75	2·55
br-C_{27}	1·05	2·35	1·26
i-C_{27}	1·26	0·63	0·43
n-C_{27}	7·29	14·86	9·90
br-C_{28}	1·01	0·84	3·44
a-C_{28}	0·99	0·68	1·62
n-C_{28}	2·70	2·70	3·40

n = normal; i = *iso*-branched; a = ante*iso* branched; br = unknown type of branching; ? = unknown.

(*continued overleaf*)

TABLE 2—*continued*

	Males	Female	Eggs
br-C_{29}	0·75	Trace	0·70
i-C_{29}	0·56	2·27	4·02
n-C_{29}	21·67	32·10	7·70
br-C_{30}	2·12	3·08	4·61
a-C_{30}	0·79	0·76	1·32
n-C_{30}	2·64	1·26	2·26
br-C_{31}	0·60	0·69	1·24
i-C_{31}	0·58	0·58	0·82
n-C_{31}	5·40	6·66	3·44
br-C_{32}	0·50	1·27	1·38
a-C_{32}	0·34	0·88	0·80
n-C_{32}	1·14	0·50	0·47
br-C_{33}	0·83	0·89	0·77
i-C_{33}	0·37	0·28	0·82
?	0·23	—	1·02
n-C_{33}	1·69	1·79	0·89
br-C_{34}	0·91	0·70	0·99
a-C_{34}	0·26	0·33	0·36
n-C_{34}	1·13	0·21	0·69
br-C_{35}	1·21	0·23	0·58
i-C_{35}	1·01	0·33	Trace
?	0·76	—	—
n-C_{35}	1·20	0·39	0·82
br-C_{36}	0·81	0·40	1·30
a-C_{36}	0·91	0·58	1·30
?	—	—	0·54
n-C_{36}	0·63	0·21	—
> n-C_{36}	Trace	Trace	Trace

n = normal; i = *iso*-branched; a = ante*iso*-branched; br = unknown type of branching; ? = unknown.

such a high percentage of unsaturated hydrocarbons. In the microorganism *Sarcina lutea* 90 per cent of the total hydrocarbons are mono-unsaturated showing either an *iso* or ante*iso* type of branching (TORNABENE *et al.*, 1967, 1970; ALBRO and DITTMER, 1969). Similar results were obtained from other microorganisms of the family Micrococcaceae (TORNABENE *et al.*, 1970). The saturated hydrocarbon fractons obtained from *Graphidostreptus* were not contaminated with unsaturated components as no differences could be observed in the gas chromatograms upon hydrogenation.

After plotting the logarithm of the relative retention time against the carbon number, in all groups three parallel lines resulted: *n*-alkanes, 2-(3-)methylalkanes (*iso* and ante*iso*), and a series of alkanes with an unknown type of branching.

The composition of the saturated hydrocarbon fractions of males, females, and eggs are tabulated in Table 2, whereas the total percentages of the different homologous series are given in Table 3.

TABLE 3—TOTAL PERCENTAGES OF THE DIFFERENT HOMOLOGOUS SERIES OF SATURATED HYDROCARBONS IN MALES, FEMALES, AND EGGS OF *G. tumuliporus*

	Males		Females		Eggs	
n-Alkanes	62·78		75·22		59·24	
Even chain		16·53		12·62		20·51
Odd chain		46·25		62·60		38·73
iso- and ante*iso*-alkanes	15·42		10·14		16·89	
Even chain		7·08		4·48		7·40
Odd chain		8·34		5·66		9·49
Unknown branched-chain alkanes	19·07		13·54		20·42	
Even chain		10·16		8·24		13·99
Odd chain		8·91		5·30		6·43
Not determined	2·67		1·15		3·47	
Total even chain components	33·77		25·34		41·90	
Total odd chain components	63·50		73·56		54·65	

DISCUSSION

In males, females, and eggs of *G. tumuliporus*, *n*-alkanes with a chain length of 15 to 36 carbon atoms constitute 59·24 to 75·22 per cent of the saturated hydrocarbons. Odd chain components strongly predominate over even chain ones (Table 3), *n*-heptacosane, *n*-nonacosane, and *n*-hentriacontane being the most abundant components in males and females, whereas in eggs *n*-heptacosane is predominant, but its percentage is relatively low.

As the distribution of *n*-alkanes over the various carbon numbers in males and females strongly resembles that of plant straight-chain hydrocarbons (KOLATTUKUDY, 1970) and differs from the unsaturated ones in *Graphidostreptus* (Oudejans, unpublished results) it is possible that most of the *n*-alkanes originate from the food.

Rather large amounts of short-chain hydrocarbons occur in the eggs, in comparison with those of the female specimens. Besides *n*-alkanes, several branched-chain homologous series of hydrocarbons occur in males, females, and eggs. Both 2-methyl (*iso*) and 3-methyl (ante*iso*) alkanes are present. If occurring together *iso* and ante*iso* hydrocarbons are hardly separable from each other by gas–liquid chromatography (JAROLÍMEK *et al.*, 1964; STRÁNSKÝ *et al.*, 1970), only with Apiezon L as the stationary phase can a good separation be obtained (STREIBL and KONEČNÝ, 1967; WOLLRAB *et al.*, 1967; BRIESKORN and BECK, 1969).

In females and eggs 2-methyloctacosane (*iso*-C_{29}) predominates, whereas in the males no distinctly predominant component is present.

In the range C_{15} to C_{23} *iso* and ante*iso* alkanes occur together and from C_{24} on the even chain components possess an ante*iso* structure, whilst the odd chain ones are *iso*-branched.

Even chain ante*iso* and odd chain *iso* hydrocarbons have been observed in several plants (MOLD *et al.*, 1963; BRIESKORN and FEILNER, 1968; KOLATTUKUDY, 1970), whereas in animals usually only the *iso* (HUTCHINS and MARTIN, 1968) or ante*iso* (ARMOLD *et al.*, 1969; TARTIVITA and JACKSON, 1970) components are present.

The third branched-chain series shows retention times of internally branched monomethylalkanes or dimethylalkanes. The equivalent chain length (ECL) values (MIWA, 1963) of this series were between 0·20 and 0·42 higher than the corresponding straight-chain components. According to BRIESKORN and BECK (1970) this value is 0·30 to 0·35 for internally branched monomethylalkanes and 0·21 for 2,(ω-1)-dimethylalkanes. The rather variable values of these branched-chain hydrocarbons of *G. tumuliporus* may indicate that several types of branched-chain alkanes are present in this series. These unknown branched-chain hydrocarbons constitute 19·07, 13·54, and 20·42 per cent of the saturated hydrocarbons from males, females, and eggs, respectively. In all three groups the C_{30} homologue is the most abundant one.

Also in the hydrocarbons of the land snail *Cepaea nemoralis* we reported a series of branched-chain components with similar retention times (VAN DER HORST and OUDEJANS, 1972). Furthermore, a few unknown components are present, indicated in Table 2 with a '?'.

It is likely that apart from the unknown branched-chain series, *n*-alkanes, *iso*-, and ante*iso*-alkanes are partly derived from the food, because their distributions resemble those from plants. In experiments with radioactive precursors we hope to solve this problem.

Because of their very low amounts the true nature of the unknown branched-chain hydrocarbons has to be determined by means of a combined gas–liquid chromatography–mass spectrometry technique.

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