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CUTICULAR HYDROCARBONS OF ADULT *ONYMACRIS BICOLOR* (HAAG) AND *ONYMACRIS BOSCHIMANA* (PÉRINGUEY) (COLEOPTERA: TENEBRIONIDAE)

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Abstract—1. The cuticular hydrocarbons of *O. bicolor* (OBC) and *O. boschimana* (OBS) comprise *n*-alkanes (OBC, nC_{19}–nC_{30}, 31.0%; OBS, nC_{20}–nC_{30}, 22.8%), 3-methylalkanes (OBC, C_{24}–C_{28}, approx. 15.6%; OBS, C_{24}–C_{30}, 23.7%), internally branched monomethylalkanes (OBC, C_{23}–C_{32}, 29.8%; OBS, C_{25}–C_{32}, approx. 34.3%), dimethylalkanes (OBC, C_{23}–C_{39}, approx. 19.8%; OBS, C_{27}–C_{33}, 18.6%) and trimethylalkanes (OBC, C_{26}–C_{41}, 1.4%; OBS, C_{32} and C_{34}, 0.2%). Unusual 3,5-dimethylalkanes (C_{29} and C_{31}) were identified in OBS and tetramethylalkanes (C_{39}, C_{40} and C_{42}) were tentatively identified in OBC (total 2.4%).

2. OBC and OBS have many, though not all, of the hydrocarbon characteristics of tribe, Adesmiini. Of the hydrocarbon characteristics of genus *Onymacris*, OBC and OBS have high proportions of 3-methylheptacosane and 3-methynonacosane respectively, though neither species has nC_{31} and the problem remains of characterizing the genus and separating it from other closely-related genera.

3. The hydrocarbon compositions of five congeneric species reflect the subdivision of genus *Onymacris*. Group 1 species, OBS and OR (*O. rugatipennis*) have fewer hydrocarbons with a narrower chain length range than group 2 species, OP (*O. plana*), OM (*O. marginipennis*) and OBC and their hydrocarbon mixtures contain a few components in high abundance rather than several components in moderate abundance as in group 2 species. The close relationship between the "white" *Onymacris* species OM and OBC is reflected in their hydrocarbon compositions which show marked qualitative and quantitative similarities.

4. Dimethylalkane biosynthesis in OBC is examined. Slight changes in the incorporation sequence of derivatives during chain elongation can affect branch position and chain length. The early incorporation of branch donors during chain elongation results in the biosynthesis of dimethylalkanes with variable branching patterns.

5. OBS, an inland species, has a hydrocarbon mixture with a higher and narrower melting range than OBC, a coastal species. The composition and melting characteristics of hydrocarbon mixtures are considered in relation to their proposed function as a fluid matrix and to the range of environmental temperatures encountered by species.

INTRODUCTION

Cuticular hydrocarbons are components of the superficial layer of lipid which occurs in an insect's epicuticle (Hepburn, 1985). Hydrocarbon composition varies among species and the taxonomic value of hydrocarbon composition is currently being investigated in family Tenebrionidae (Lockey and Metcalfe, 1988), a diverse family of beetles comprising some 14,000 species divided into 92 tribes (Gebien, 1937) and subfamilies, Tenebrioninae and Tentyriinae (Koch, 1955).

So far in the survey, in which samples of the naturally occurring populations of selected species are investigated by a set procedure, 22 species representing 10 tribes and including 5 sets of congenerics have been examined. Results show that hydrocarbon composition and taxonomic grouping within family Tenebrionidae are related to the extent that (a) hydrocarbon composition is species-specific, (b) closely related species such as congenerics tend to have hydrocarbon mixtures which are qualitatively similar and quantitatively dissimilar whereas distantly related species tend to have mixtures which are both qualitatively and quantitatively dissimilar, (c) species belonging to higher taxa such as genus and tribe tend to have hydrocarbon mixtures with shared characteristics and (d) hydrocarbon composition tends to reflect relationships between genera or tribes (Lockey and Metcalfe, 1988).

Currently the hydrocarbon compositions of three species of genus *Onymacris* tribe Adesmiini from Namibia (Penrith, 1975, 1979, 1984, 1986) namely, *O. plana*, *O. rugatipennis* (Lockey, 1982a) and *O. marginipennis* (Lockey, 1982b) have been determined. In this work, qualitative and quantitative differences in hydrocarbon composition clearly distinguished *O. marginipennis* from *O. plana* and *O. rugatipennis*, while quantitative differences separated the latter two species. However, it proved difficult to characterize genus *Onymacris* on the basis of hydrocarbon composition and to separate it from closely related genera such as *Physadesmia* (Lockey, 1982c) and *Metriopus* (Lockey, 1984a).

In the work to be described, the hydrocarbon composition of two other species of genus *Onymacris*, namely *O. bicolor* and *O. boschimana* have been determined and the relationships within genus *Onymacris* and tribe Adesmiini examined further.

MATERIALS AND METHODS

Organic solvents of the "AnalaR" grade of purity were used throughout the work after distillation through a 100 cm fractionating column.

Live adults of *Onymacris bicolor* (OBC) and *O. boschimana* (OBS) were supplied by the Desert Ecological Research Unit at Gobabeb, Namibia. Both species were killed by freezing at −20°C and the cuticular lipid of each was extracted from the whole bodies of both males and females by refluxing in chloroform for 1 hr. The insect–chloroform volume ratio was approximately 1:5. The solutions were filtered, dried with anhydrous sodium sulphate, refiltered and evaporated to dryness without heating with a model RE111 Buchi Rotavapor. Each weighed lipid residue (OBC, 0.39673 g; OBS, 0.29321 g) was then dissolved in a minimum volume of petroleum spirit (boiling range 60–70°C), applied to a 200 × 20 mm i.d. glass column packed with alumina (Merck, neutral) and eluted with petroleum spirit until no further hydrocarbons were deposited (OBC, 0.02693 g; OBS, 0.03536 g). Before use, the alumina was heated at 110°C for 2 hr and transferred to petroleum spirit while still hot.

The hydrocarbon mixtures were analysed with a Perkin–Elmer model 8500 gas chromatograph with integration facilities using a 30 m J&W fused silica capillary column (i.d. 0.258 m) coated with non-polar surface-bonded phase DB-1 (0.1 μm thick). Helium at 0.8 ml/min was used as the carrier gas and the split ratio was approximately 1:30. The temperature of the injector and the FID detector was 350°C. At first the mixtures were analysed by temperature-programming from 50 to 320°C at 5°C/min. In subsequent analyses the loss of high molecular weight components was minimized by injecting samples with the column held at 100°C for 2 min with the splitter closed, followed by heating the column from 100 to 150°C at 20°C/min and from 150 to 320°C at 2°C/min with the splitter open. The oven was kept at 320°C for 5 min in the case of the OBC sample because of the presence of high molecular weight components. Analytical data were stored on hard disk. Retention indices (I) were calculated from retention times recorded by the model 8500 gas chromatograph. For the determination of retention indices each sample was co-injected with a mixture of even-numbered *n*-alkanes ranging from nC_{18} to nC_{42} and temperature-programmed from 100 to 320°C at 2°C/min (Kováts, 1965).

Methylalkanes were separated from straight-chain components by refluxing each mixture with Linde molecular sieve (type 5A, 1.5 mm pellets) in *iso*-octane for 8 hr. Before use, the molecular sieve was heated at 380°C in a stream of nitrogen for 48 hr and added while still hot to the hydrocarbon mixture dissolved in *iso*-octane (O'Connor *et al.*, 1962).

Methylalkanes were identified by their electron impact (EI) and chemical ionization (CI, *iso*-butane) mass spectra which were obtained with a model 70-250S VG Analytical gas chromatograph–mass spectrometer using a 25 m capillary column similar to the one used in GC analyses. Samples were analysed by the procedure used in GC analysis. The temperature of the ion source was 240°C and the ionization voltage, 70 eV. Helium at a linear velocity of 60 cm/min was used as the carrier gas. EI and CI mass spectral scans (MS scans), ranging from m/z 700 to m/z 20, were taken alternately at a scan rate of 1 sec/decade. Interscan time was 0.3 sec and resolution 1000. MS scans were selected for examination from chromatograms generated by the data system of the model 70-250S, including the total ion count (TIC) chromatogram and the m/z 57, m/z 99 and m/z 113 mass chromatograms. Background was subtracted from MS scans before they were examined.

The mass spectra of methylalkanes were interpreted according to the criteria proposed by McCarthy *et al.* (1968), Nelson (1978), Nelson *et al.* (1972, 1981) and Pomonis *et al.* (1978, 1980).

RESULTS

The gas chromatograms of the hydrocarbon mixtures of *O. bicolor* and *O. boschimana* are shown in Fig. 1 while Tables 1 and 2 give the identity and the approximate percentage composition of components obtained by peak integration.

Both mixtures lack unsaturated hydrocarbons and comprise the following hydrocarbon classes: A, *n*-alkanes; C2, 3-methylalkanes, D, internally branched monomethylalkanes, E, dimethylalkanes and F, trimethylalkanes. *O. bicolor*, in addition has a low proportion of components tentatively identified as tetramethylalkanes (class G) (Fig. 2).

Class A: n-*alkanes*

The two species have different proportions of *n*-alkanes (OBC, 31.0%; OBS, 22.8%). *O. bicolor* has a complete homologous series of *n*-alkanes ranging from nC_{19} to nC_{30}. nC_{27} (GC peak OBC43, 10.1%) and nC_{29} (GC peak OBC62, 10.8%) are the most abundant components of the series. *O. boschimana* has a similar series ranging from nC_{20} to nC_{30} in which nC_{29} (GC peak OBS29, 17.8%) is the most abundant component.

Class C2: 3-*methylalkanes*

This class accounts for approximately 15.6 and 23.7% of the hydrocarbons of *O. bicolor* and *O. baschimana*, respectively. Both species have discontinuous series of 3-methylalkanes. In *O. bicolor* the series ranges in chain length from C_{23} to C_{27} with the 3-methylisomer of C_{27} (GC peak OBC49, 13.8%) in the highest abundance, whereas in *O. boschimana* 3-methylalkanes range in chain length from C_{23} to C_{29} with 3-methylnonacosane (GC peak OBS34, 20.7%) the most abundant.

Class D: *internally branched monomethylalkanes*

Components of class D account for approximately 29.8% and 34.3% of the hydrocarbon mixtures of *O. bicolor* and *O. boschimana*, respectively. The class D hydrocarbons of *O. bicolor* form a continuous series ranging in chain length from C_{22} to C_{31}. The monomethylisomers of C_{27} are the most abundant (10.1%) with the monomethylisomers of C_{25} (5.6%), C_{28} (4.0%) and C_{29} (6.3%) also in relatively high proportions. The continuous series of class D hydrocarbons in *O. boschimana* is less extensive than that of *O. bicolor* ranging in chain length from C_{24} to C_{31}. The monomethylisomers of C_{27} (13.6%) and C_{29} (17.2%) are the main components of the series.

Both species have homologous series of 4-methyl- and 6-methylalkanes with even chains and 5-methyl- and 7-methylalkanes mainly with odd chains. Those monomethylalkanes with their branches centrally positioned from carbon 9 to 15 of the alkyl chain, form an extensive series in *O. bicolor* ranging in chain length from C_{23} to C_{31}, whereas in *O. boschimana* centrally branched monomethylalkanes are restricted to chain lengths ranging from C_{27} to C_{31}.

Class E: *dimethylalkanes*

Dimethylalkanes account for approximately 19.8% and 18.6% of the hydrocarbons of *O. bicolor* and *O. boschimana*, respectively. The dimethylalkanes of

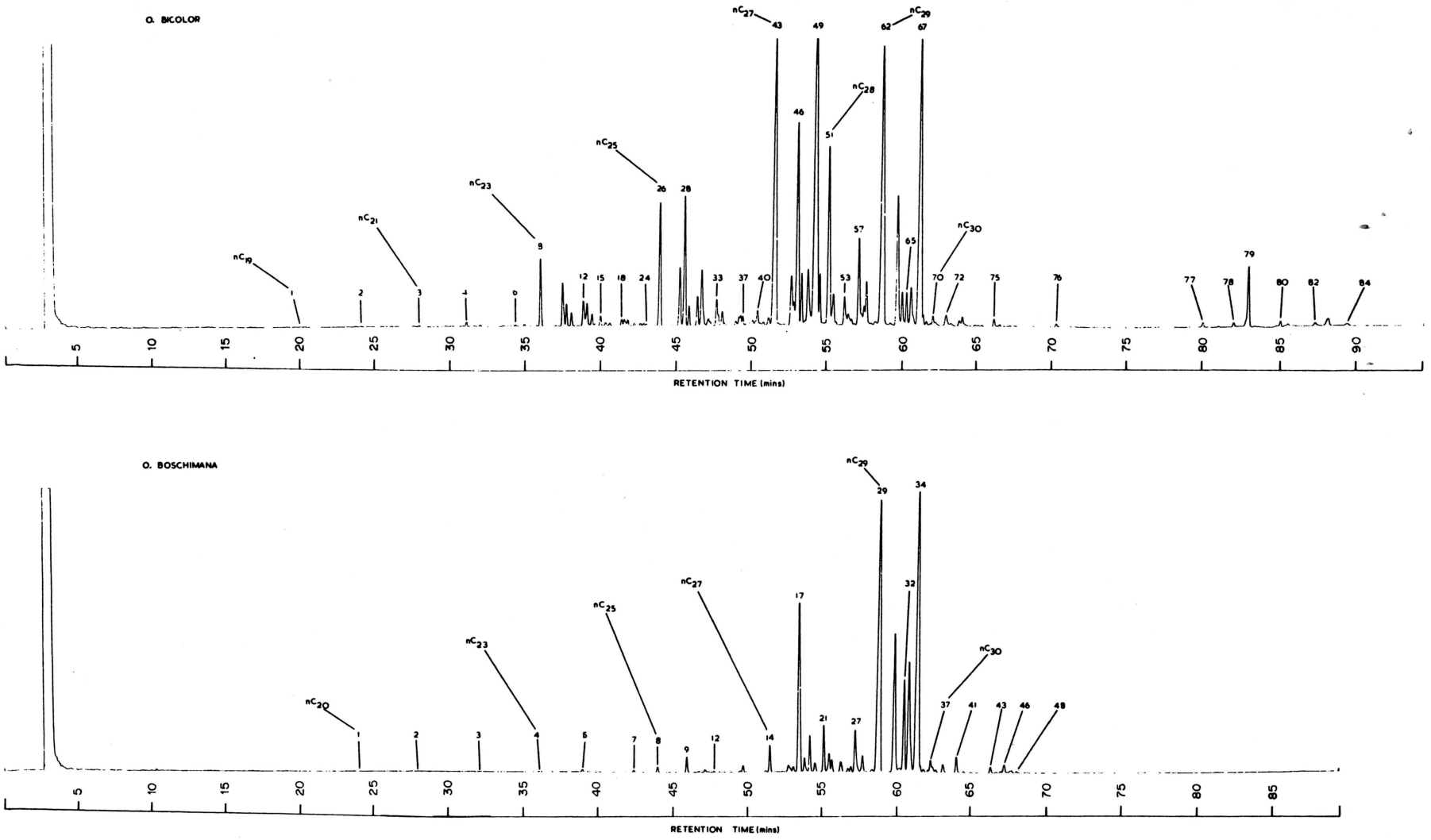

Fig. 1. Gas chromatograms of the hydrocarbons of *O. bicolor* and *O. boschimana*. GC analyses on a 30 m fused silica capillary column with surface bonded phase DB-1. Column held at 100°C with the splitter closed for 2 min, followed by temperature programming from 100 to 150°C at 20°C/min and from 150 to 320°C at 2°C/min with the splitter open. Carrier gas: helium at 0.8 ml/min.

Table 1. Hydrocarbons of *O. bicolor* (retention indices and percentage composition values are the average of three replicates)

GC peak No.	Retention index (*I*)	Composition (%)	Hydrocarbon	Diagnostic fragment ions of methylalkanes (*m/z*)
OBC				
1	1900	t	*n*-Nonadecane	
2	2000	t	*n*-Eicosane	
3	2100	t	*n*-Heneicosane	
4	2176	0.1	7,11-Dimethylheneicosane	112/3, 168/9, 183, 239, 324(M^+)
5	2200	0.1	*n*-Docosane	
6	2258	t	7-, 8-, 9-, 10- & 11-Methyldocosane	112/3, 126/7, 140/1, 154/5, 168/9, 182/3, 196/7, 210/1, 224/5, 238/9, 324(M^+)
7	2273	0.1	4-Methyldocosane	70/1, 252/3, 280/1, 324(M^+)
8	2300	1.6	*n*-Tricosane	
9	2337	1.2	9- & 11-Methyltricosane	140/1, 168/9, 196/7, 224/5, 338(M^+)
10	2343	0.5	7-Methyltricosane	112/3, 224/5, 252/3, 338(M^+)
11	2351	0.3	5-Methyltricosane	84/5, 252/3, 280/1, 338(M^+)
12	2371	0.7	9,13-Dimethyltricosane	140/1, 168/9, 211, 239, 352(M^+)
13	2377	0.7	3-Methyltricosane	56/7, 280/1, 308/9, 352(M^+)
			7,13-Dimethyltricosane	112/3, 168/9, 211, 267, 352(M^+)
14	2385	0.3	5,9-Dimethyltricosane	84/5, 155, 224/5, 295, 352(M^+)
15	2400	0.2	*n*-Tetracosane	
16	2408	0.1	3,13-Dimethyltricosane	56/7, 168/9, 211, 323, 352(M^+)
17	2416	0.1	5,9,13-Trimethyltricosane	84/5, 155, 168/9, 225, 239, 309, 366(M^+)
18	2436	0.2	9-, 10-, 11- & 12-Methyltetracosane	140/1, 154/5, 168/9, 182/3, 196/7, 210/1, 224/5, 238/9, 352(M^+)
19	2442	0.2	7-Methyltetracosane	112/3, 238/9, 266/7, 352(M^+)
20	2446	0.1	6-Methyltetracosane	98/9, 252/3, 280/1, 352(M^+)
21	2458	0.1	4-Methyltetracosane	70/1, 280/1, 308/9, 352(M^+)
22	2468	0.1	10,14-Dimethyltetracosane	154/5, 168/9, 225, 239, 366(M^+)
23	2474	0.1	7,11-Dimethyltetracosane	112/3, 183, 210/1, 281, 366(M^+)
24	2478	t	6,10-Dimethyltetracosane	98/9, 169, 224/5, 295, 366(M^+)
25	2493	t	4,14-Dimethyltetracosane	70/1, 168/9, 225, 323, 366(M^+)
26	2500	3.5	*n*-Pentacosane	
		t	6,10,14-Trimethyltetracosane	98/9, 168/9, 239, 309, 380(M^+)
27	2536	1.6	11- & 13-Methylpentacosane	168/9, 196/7, 224/5, 366(M^+)
28	2544	3.6	7-Methylpentacosane	112/3, 252/3, 280/1, 366(M^+)
29	2551	0.4	5-Methylpentacosane	84/5, 280/1, 308/9, 366(M^+)
30	2566	0.7	11,15-Dimethylpentacosane	168/9, 239, 380(M^+)
31	2573	1.8	7,15- & 7,17-Dimethylpentacosane	112/3, 140/1, 168/9, 239, 267, 295, 380(M^+)
			3-Methylpentacosane	56/7, 308/9, 336/7, 366(M^+)
32	2585	0.2	5,15-Dimethylpentacosane	84/5, 168/9, 239, 323, 380(M^+)
33	2600	0.7	*n*-Hexacosane	
		t	7,11,15-Trimethylpentacosane	112/3, 168/9, 183, 239, 253, 309, 394(M^+)
34	2608	0.4	3,7-Dimethylpentacosane	56/7, 127, 280/1, 351, 380(M^+)
35	2633	0.1	12- & 13-Methylhexacosane	182/3, 196/7, 210/1, 224/5, 380(M^+)
36	2640	0.2	7- & 9-Methylhexacosane	112/3, 140/1, 266/7, 294/5, 380(M^+)
37	2645	0.2	6-Methylhexacosane	98/9, 280/1, 308/9, 380(M^+)
38	2656	0.1	4-Methylhexacosane	70/1, 308/9, 336/7, 380(M^+)
39	2662	0.2	10,14- & 11,15-Dimethylhexacosane	154/5, 168/9, 182/3, 196/7, 225, 239, 253, 267, 394(M^+)
40	2671	0.5	3-Methylhexacosane	56/7, 322/3, 350/1, 380(M^+)
41	2681	0.1	5,9-Dimethylhexacosane	84/5, 155, 266/7, 337, 394(M^+)
42	2691	0.2	4,8-Dimethylhexacosane	70/1, 141, 280/1, 351, 394(M^+)
43	2700	10.1	*n*-Heptacosane	
		t	3,7- & 3,9-Dimethylhexacosane	56/7, 127, 155, 266/7, 294/5, 365, 394(M^+)
44	2731	1.6	11- & 13-Methylheptacosane	168/9, 196/7, 224/5, 252/3, 394(M^+)
45	2735	0.6	9-Methylheptacosane	140/1, 252/3, 280/1, 394(M^+)
46	2743	6.8	7-Methylheptacosane	112/3, 280/1, 308/9, 394(M^+)
47	2749	1.1	5-Methylheptacosane	84/5, 308/9, 336/7, 394(M^+)
48	2761	1.8	11,15-Dimethylheptacosane	168/9, 196/7, 239, 267, 408(M^+)
49	2773	13.8	3-Methylheptacosane	56/7, 336/7, 364/5, 394(M^+)
50	2781	1.0	5,9-Dimethylheptacosane	84/5, 155, 280/1, 351, 408(M^+)
51	2800	3.9	*n*-Octacosane	
		t	7,11,15- & 9,13,17-Trimethylheptacosane	112/3, 140/1, 168/9, 183, 196/7, 211, 239, 253, 267, 281, 309, 337, 422(M^+)
52	2807	1.0	3,7- & 3,9-Dimethylheptacosane	56/7, 127, 155, 280/1, 308/9, 379, 408(M^+)
53	2828	0.9	10-, 11-, 12-, 13- & 14-Methyloctacosane	154/5, 168/9, 182/3, 196/7, 210/1, 224/5, 238/9, 252/3, 266/7, 280/1, 408(M^+)
54	2835	0.4	8-Methyloctacosane	126/7, 280/1, 308/9, 408(M^+)
55	2842	0.2	6-Methyloctacosane	98/9, 308/9, 336/7, 408(M^+)
56	2851	t	5-Methyloctacosane	84/5, 322/3, 350/1, 408(M^+)
57	2857	2.5	4-Methyloctacosane	70/1, 336/7, 364/5, 408(M^+)
58	2861	0.3	8,12,16-Trimethyloctacosane	126/7, 196/7, 267, 337, 436(M^+)
59	2866	0.5	8,14,18- & 8,14,20-Trimethyloctacosane	126/7, 140/1, 168/9, 225, 239, 295, 323, 337, 436(M^+)
60	2871	1.2	5,9-, 5,11- & 5,13-Dimethyloctacosane	84/5, 155, 183, 211, 238/9, 266/7, 294/5, 365, 422(M^+)
61	2894	0.1	4,8-, 4,10- & 4,12-Dimethyloctacosane	70/1, 141, 169, 197, 252/3, 280/1, 308/9, 379, 422(M^+)
62	2900	10.8	*n*-Nonacosane	
63	2931	4.4	11-, 13- & 15-Methylnonacosane	168/9, 196/7, 224/5, 252/3, 280/1, 422(M^+)
64	2941	1.0	7-Methylnonacosane	112/3, 308/9, 336/7, 422(M^+)
65	2950	0.9	5-Methylnonacosane	84/5, 336/7, 364/5, 422(M^+)
66	2959	1.5	9,13-, 11,15- & 13,17-Dimethylnonacosane	140/1, 168/9, 196/7, 211, 224/5, 239, 252/3, 267, 295, 323, 436(M^+)
67	2977	7.9	7,17-Dimethylnonacosane	126/7, 196/7, 267, 351, 436(M^+)

Table 1—*continued*

GC peak No.	Retention index (*I*)	Composition (%)	Hydrocarbon	Diagnostic fragment ions of methylalkanes (*m*/*z*)
68	2983	0.2	5,9-Dimethylnonacosane	84/5, 155, 308/9, 379, 436(M^+)
69	2989	0.1	9,13,17-Trimethylnonacosane	140/1, 196/7, 211, 267, 281, 337, 450(M^+)
70	3000	0.1	*n*-Triacontane	
71	3008	0.3	3,15-Dimethylnonacosane	56/7, 224/5, 239, 407, 436(M^+)
72	3029	0.3	11-, 13-, 14-, & 15-Methyltriacontane	168/9, 196/7, 210/1, 224/5, 238/9, 252/3, 266/7, 294/5, 436(M^+)
73	3057	0.2	11,15- & 13,17-Dimethyltriacontane	168/9, 196/7, 210/1, 238/9, 267, 281, 309, 450(M^+)
74	3062	0.3	11,15,19-Trimethyltriacontane	168/9, 182/3, 239, 253, 309, 323, 464(M^+)
75	3128	0.2	11-, 13- & 15-Methylhentriacontane	168/9, 196/7, 224/5, 252/3, 280/1, 308/9, 450(M^+)
76	3259	t	9,13-Dimethyldotriacontane	140/1, 211, 294/5, 365, 478(M^+)
77	3584	0.1	8,14,18,22-Tetramethylpentatriacontane	126/7, 210/1, 225, 281, 295, 351, 365, 449, 548(M^+)
78	3651	0.1	—	
79	3687	1.9	8,14,18,22-Tetramethylhexatriacontane	126/7, 224/5, 295, 365, 463, 562(M^+)
80	3764	0.1	7,15- & 7,17-Dimethylheptatriacontane	112/3, 239, 267, 308/9, 336/7, 463, 548(M^+)
81	3783	t	—	
82	3850	0.1	9,15,19-Trimethyloctatriacontane	140/1, 239, 294/5, 309, 365, 463, 576(M^+)
83	3882	0.4	8,14,18,22-Tetramethyloctatriacontane	126/7, 224/5, 295, 365, 463, 590(M^+)
84	3929	t	—	

Table 2. Hydrocarbons of *O. boschimana* (retention indices and percentage composition values are the average of three replicates)

GC peak No.	Retention index (*I*)	Composition (%)	Hydrocarbon	Diagnostic fragment ions of methylalkanes (*m*/*z*)
OBS				
1	2000	t	*n*-Eicosane	
2	2100	t	*n*-Heneicosane	
3	2200	t	*n*-Docosane	
4	2300	t	*n*-Tricosane	
5	2369	0.1	3-Methyltricosane	56/7, 280/1, 308/9, 338(M^+)
6	2400	t	*n*-Tetracosane	
7	2456	0.1	4-Methyltetracosane	70/1, 280/1, 308/9, 352(M^+)
8	2500	0.3	*n*-Pentacosane	
9	2550	0.8	5-Methylpentacosane	84/5, 280/1, 308/9, 366(M^+)
10	2570	t	3-Methylpentacosane	56/7, 308/9, 336/7, 366(M^+)
11	2582	t	5,9-Dimethylpentacosane	84/5, 155, 252/3, 323, 380(M^+)
12	2600	t	*n*-Hexacosane	
		t	3,7-Dimethylpentacosane	56/7, 127, 280/1, 351, 380(M^+)
13	2650	0.4	5- & 6-Methylhexacosane	84/5, 98/9, 280/1, 294/5, 308/9, 322/3, 380(M^+)
14	2700	1.4	*n*-Heptacosane	
		t	3,7- & 4,8-Dimethylhexacosane	56/7, 70/1, 127, 141, 280/1, 294/5, 351, 365, 394(M^+)
15	2735	0.5	9- & 11-Methylheptacosane	140/1, 168/9, 224/5, 252/3, 280/1, 394(M^+)
16	2741	0.3	7-Methylheptacosane	112/3, 280/1, 308/9, 394(M^+)
17	2754	12.8	5-Methylheptacosane	84/5, 308/9, 336/7, 394(M^+)
18	2762	0.6	11,15-Dimethylheptacosane	168/9, 196/7, 239, 267, 408(M^+)
19	2772	1.9	3-Methylheptacosane	56/7, 336/7, 364/5, 394(M^+)
20	2781	0.5	5,9-Dimethylheptacosane	84/5, 155, 280/1, 351, 408(M^+)
21	2800	2.6	*n*-Octacosane	
22	2808	1.2	3,7-, 3,9-, 3,11- & 3,13-Dimethylheptacosane	56/7, 127, 155, 183, 211, 224/5, 252/3, 280/1, 308/9, 379, 408(M^+)
23	2813	0.7	3,5-Dimethylheptacosane	56/7, 99, 336/7, 379, 408(M^+)
24	2830	0.6	11-, 13- & 14-Methyloctacosane	168/9, 196/7, 210/1, 224/5, 238/9, 266/7, 408(M^+)
25	2844	0.2	6-Methyloctacosane	98/9, 308/9, 336/7, 408(M^+)
26	2849	0.3	5-Methyloctacosane	84/5, 322/3, 350/1, 408(M^+)
27	2858	3.3	11,15- & 12,16-Dimethyloctacosane	168/9, 182/3, 196/7, 210/1, 239, 253, 267, 281, 422(M^+)
28	2872	1.1	3-Methyloctacosane	56/7, 350/1, 378/9, 408(M^+)
29	2900	17.8	*n*-Nonacosane	
		t	4,8-Dimethyloctacosane	70/1, 141, 308/9, 379, 422(M^+)
30	2933	10.8	11-, 13- & 15-Methylnonacosane	168/9, 196/7, 224/5, 252/3, 280/1, 422(M^+)
31	2943	0.4	7-Methylnonacosane	112/3, 308/9, 336/7, 422(M^+)
32	2953	6.0	5-Methylnonacosane	84/5, 336/7, 364/5, 422(M^+)
33	2961	9.7	11,15- & 13,17-Dimethylnonacosane	168/9, 196/7, 224/5, 239, 267, 295, 436(M^+)
34	2978	20.7	3-Methylnonacosane	56/7, 364/5, 392/3, 422(M^+)
35	2983	0.2	5,13- 5,15- & 5,17-Dimethylnonacosane	84/5, 196/7, 211, 224/5, 239, 252/3, 267, 379, 436(M^+)
36	2989	0.2	9,13,17-Trimethylnonacosane	140/1, 196/7, 211, 267, 281, 337, 436(M^+)
37	3000	0.7	*n*-Triacontane	
38	3003	0.4	3,7-, 3,9-, 3,11-, 3,13-, 3,15- & 3,17-Dimethylnonacosane	56/7, 127, 155, 183, 196/7, 211, 224/5, 239, 252/3, 267, 280/1, 308/9, 336/7, 407, 436(M^+)
39	3007	0.2	3,5-Dimethylnonacosane	56/7, 99, 364/5, 407, 436(M^+)
40	3029	0.7	8-, 10-, 12-, 13-, 14- & 15-Methyltriacontane	126/7, 154/5, 182/3, 196/7, 210/1, 224/5, 238/9, 252/3, 266/7, 280/1, 308/9, 336/7, 436(M^+)
41	3058	1.0	12,16- & 13,17-Dimethyltriacontane	182/3, 196/7, 210/1, 224/5, 253, 267, 281, 450(M^+)
42	3072	t	4,14-Dimethyltriacontane	70/1, 225, 252/3, 407, 450(M^+)
43	3129	0.4	13- & 15-Methylhentriacontane	196/7, 224/5, 252/3, 280/1, 450(M^+)
44	3140	t	—	
45	3150	0.1	—	
46	3157	0.6	11,15- & 13,17-Dimethylhentriacontane	168/9, 196/7, 224/5, 239, 252/3, 267, 295, 323, 464(M^+)
47	3171	0.2	7,15- & 7,17-Dimethylhentriacontane	112/3, 224/5, 239, 252/3, 267, 379, 464(M^+)
48	3183	t	11,15,19-Trimethylhentriacontane	168/9, 196/7, 239, 267, 309, 337, 478(M^+)

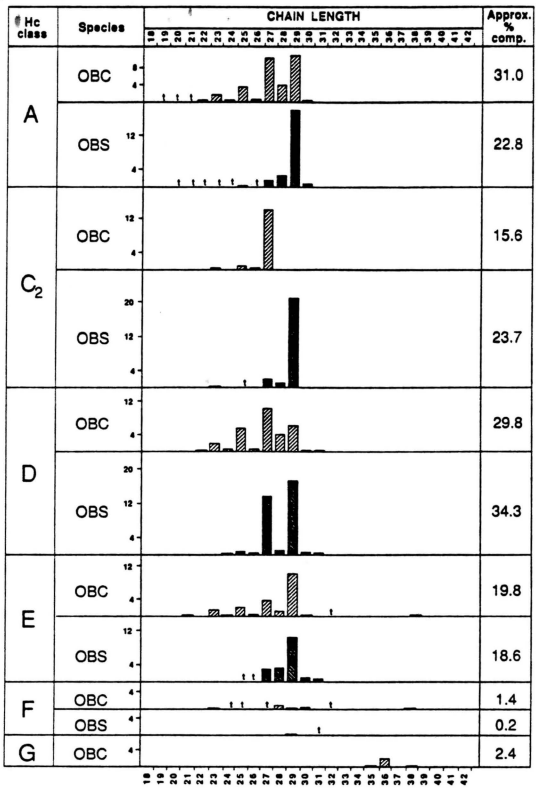

Fig. 2. Approximate percentage composition of the hydrocarbons of *O. bicolor* (OBC) and *O. boschimana* (OBS). Class A, *n*-alkanes; C2, 3-methylalkanes; D, internally branched monomethylalkanes; E, F and G, dimethyl-, trimethyl- and tetramethylalkanes, respectively.

O. bicolor form an extensive, though incomplete homologous series ranging in chain length from C_{21} to C_{37}. The dimethylisomers of C_{29} (9.9%) are the most abundant with the dimethylisomers of C_{25} (approx. 2.2%) and C_{27} (approx. 3.8%) also in relatively high abundance. The dimethylalkanes of *O. boschimana* form a complete though less extensive series, ranging in chain length from C_{25} to C_{31}. The dimethylisomers of C_{29} (10.5%) are the most abundant and the dimethylisomers of C_{27} (3.0%) and C_{28} (3.3%) the next most abundant dimethylalkanes. The dimethylalkanes of *O. boschimana* comprise two distinct types (Lockey, 1982a), namely type 1, which have their first branch centrally positioned at either carbon 11, 12 or 13 of the alkyl chain and separated exclusively from the second branch by three methylene groups and type 2 which have their first branch terminally positioned at either carbon 3, 4, 5 or 7 of the chain and separated from the second branch by an odd and variable number of methylene groups ranging from 3 to 11 (Table 2). In *O. boschimana* type 1 dimethylalkanes account for about 82% of the dimethylalkane mixture and range in chain length from C_{27} to C_{31} whereas type 2 dimethylalkanes form a more extensive series ranging in chain length from C_{25} to C_{31}. *O. bicolor* has a more complex dimethylalkane mixture and the distinction between types 1 and 2 dimethylalkanes is less marked. The dimethylalkanes of *O. bicolor* form a nearly complete sequence of dimethylisomers with their first branch positioned at carbon 3 through to carbon 13 of the alkyl chain. The type 2 dimethylalkanes of *O. bicolor* account for about 74% of the dimethylalkane mixture. This contrasts with approximately 18% in *O. boschimana*.

The type 2 dimethylalkanes of *O. boschimana* include the 3,5-dimethylisomers of C_{27} (GC peak OBS23) and C_{29} (GC peak OBS39), a type of dimethylalkane previously undetected in tenebrionid hydrocarbon mixtures (Lockey, 1988). An EI MS scan of OBS23 is given in Fig. 3 which shows a molecular ion at m/z 408 ($C_{29}H_{60}$), ion doublets at m/z 56/7 (C_4), m/z 308/9 (C_{22}) and m/z 336/7 (C_{24}) and enhanced fragment ions at m/z 99 (C_7) and m/z 379 (C_{27}).

Class F: trimethylalkanes

Trimethylalkanes account for about 1.4% and 0.2% of the hydrocarbon mixtures of *O. bicolor* and *O. boschimana*, respectively. *O. bicolor* has a mixture of 11 trimethylalkanes which form an incomplete series ranging in chain length from C_{23} to C_{38}. The mixture comprises a sequence of trimethylisomers with their first branch positioned at carbon 5 through to carbon 11 of the alkyl chain. Although most of the trimethylalkanes have three methylene groups separating their branches, such as for example, 5,9,13-trimethyltricosane (GC peak OBC17), three trimethylisomers, namely 8,14,18- and 8,14,20-trimethyloctacosane (GC peak OBC59) and 9,15,19-trimethyloctatriacontane (GC peak OBC82) have either three of five intervening methylene groups. An EI MS scan of GC peak OBC59 is given in Fig. 3 which shows a molecular ion at m/z 436 ($C_{31}H_{64}$), ion doublets at m/z 126/7 (C_9), m/z 140/1 (C_{10}) and m/z 168/9 (C_{12}) and enhanced fragment ions at m/z 225 (C_{16}), m/z 239 (C_{17}), m/z 295 (C_{21}), m/z 323 (C_{23}) and m/z 337 (C_{24}).

Two trimethylalkanes were identified in the hydrocarbon mixture of *O. boschimana*, namely 9,13,17-trimethylnonacosane (GC peak OBS36) and 11,15,19-trimethylhentriacontane (GC peak OBS48), both of which have their branches separated by three methylene groups.

Class G: tetramethylalkanes

Components amounting to approximately 2.4% of the hydrocarbon mixture of *O. bicolor* were tentatively identified as tetramethylalkanes. An EI MS scan of GC peak OBC79, 8,14,18,22-tetramethylhexatriacontane (Fig. 4), shows a M-15 ion a m/z 547 (C_{39}), ion doublets at m/z 126/7 (C_9) and m/z 224/5 (C_{16}) and enhanced fragment ions at m/z 295 (C_{21}), m/z 365 (C_{26}) and m/z 463 (C_{33}). The CI MS scan of GC peak OBC79 shows a molecular ion at m/z 562 ($C_{40}H_{82}$).

The tetramethylalkanes of *O. bicolor* form a short series with chain lengths C_{35}, C_{36} and C_{38} and with the first two branches of each tetramethylisomer separated by five methylene groups and the remaining branches by three methylene groups. dI is the difference between the retention index of a methylalkane and a *n*-alkane with the same number of carbons and the average dI value of these three components equals 316 which is consistent with them being tetramethylalkanes (Nelson *et al.*, 1988).

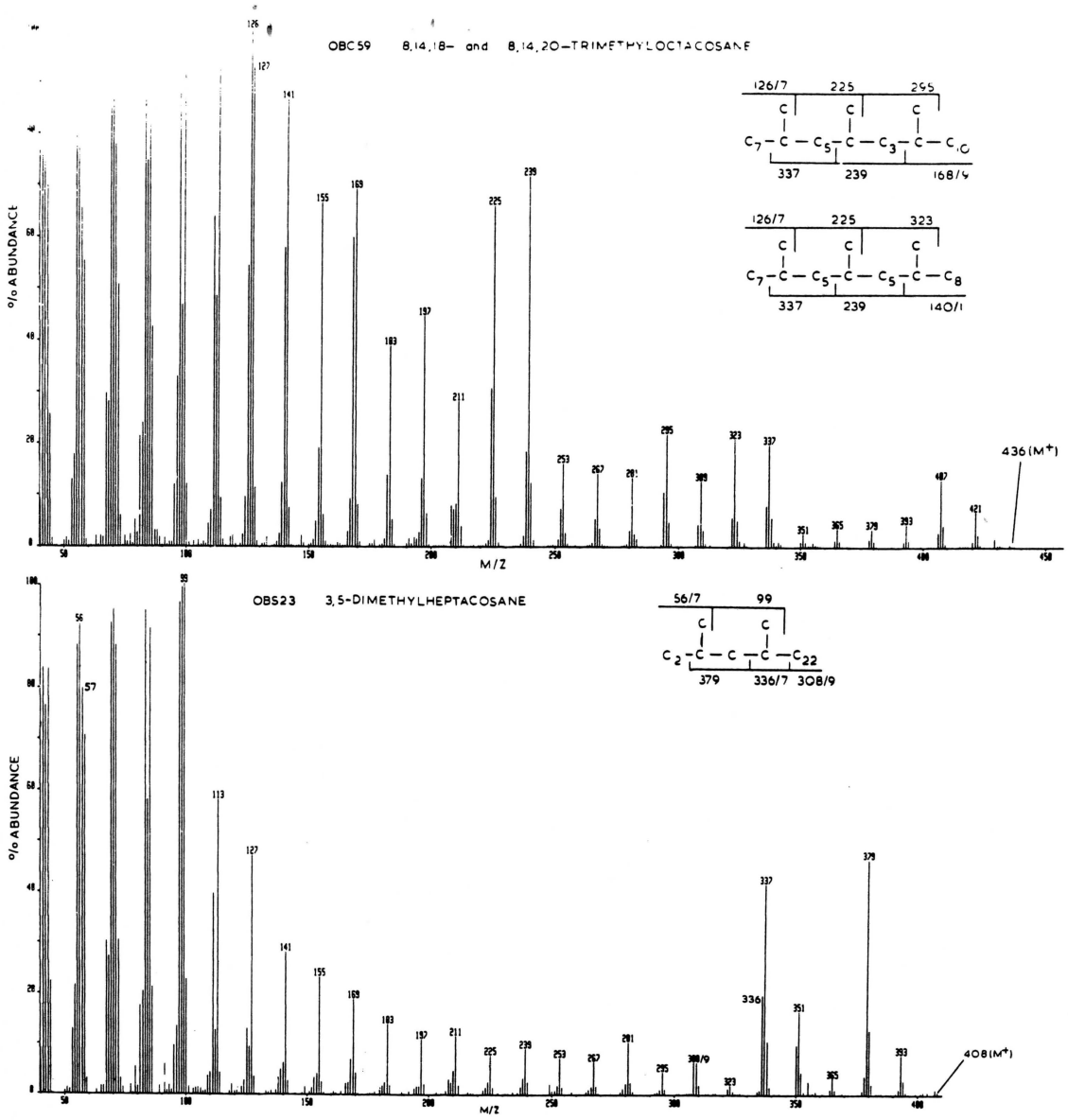

Fig. 3. EI MS scans of GC peaks OBC59 (8,14,18- and 8,14-20-trimethyloctacosane) and OBS23 (3,5-dimethylheptacosane).

DISCUSSION

The hydrocarbon mixtures of the two species show many, though not all, of the hydrocarbon characteristics of tribe Adesmiini (Lockey, 1988). Both species have high proportions of nonacosanes (OBC, 66.4%; OBS, 64.9%), though only moderate to low proportions of heptacosanes (OBC, 37.8%; OBS, 17.5%), homologous series of 4-methyl- and 5-methylalkanes and moderately to highly complex mixtures of monomethyloctacosanes and monomethyltriacontanes (OBC, 15 isomers; OBS, 10 isomers). Both species lack olefins.

Even though *O. bicolor* and *O. boschimana* are congeneric species, their hydrocarbon mixtures show marked qualitative and quantitative differences. *O. bicolor* has more cuticular hydrocarbons, particularly monomethyl- and trimethylalkanes, than *O. boschimana* (OBC, 124; OBS, 77) and its mixture has a wider chain length range (OBC, C_{19}–C_{38}; OBS, C_{20}–C_{31}). In addition, the mixture of *O. bicolor* contains several components of moderate abundance unlike that of *O. boschimana* which has a few components of high abundance (Fig. 2).

The presence of trimethylalkanes and tetramethylalkanes in *O. bicolor* and 3,5-dimethylalkanes and trimethylalkanes in *O. boschimana* separates these species from other examined tenebrionid species. However, multivariate analysis of the compositional data of 22 examined species demonstrates a clear relationship between hydrocarbon composition and tenebrionid taxa (Lockey and Metcalfe, 1988) and it may be inferred from this that the detection of these methylalkanes in *O. bicolor* and *O. boschimana* represents improved analytical technique rather than a true difference between species.

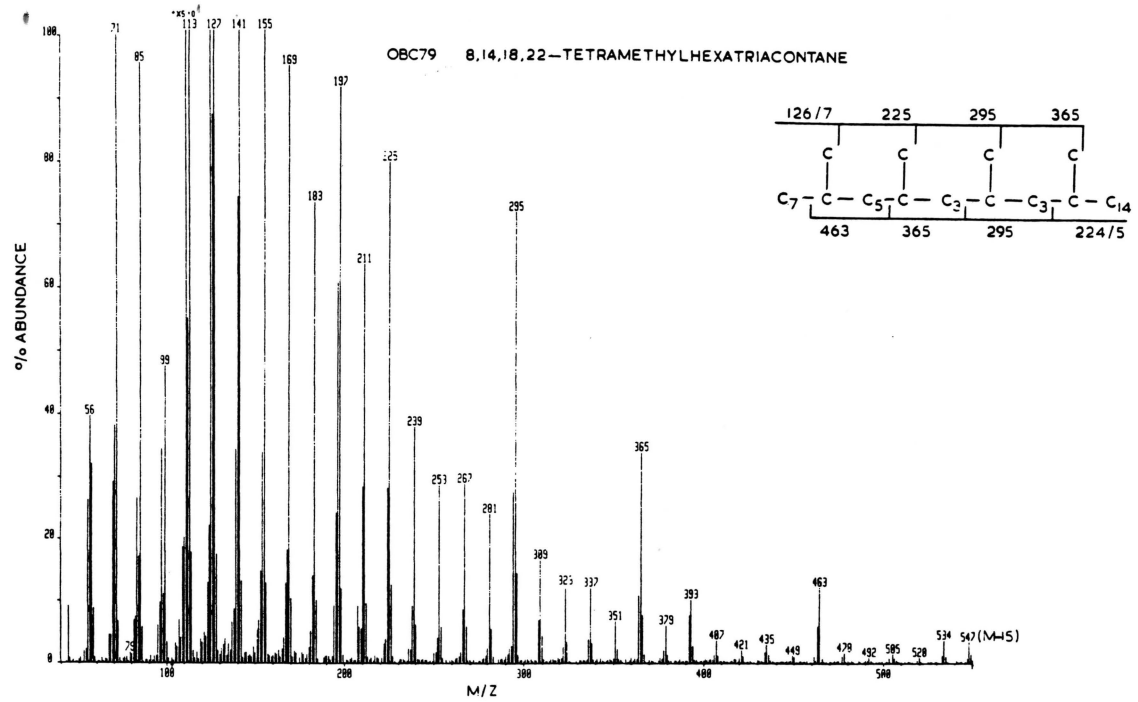

Fig. 4. EI MS scan of GC peak OBC79 (8,14,18,22-tetramethylhexatriacontane).

Trimethylalkanes have been detected in at least 17 insect species scattered among six orders (Lockey, 1988). Among the Tenebrionidae, trimethylalkanes have been found in the hydrocarbon mixtures of *Cylindrinotus laevioctostriatus* and *Phylan gibbus* (Lockey, 1981), subfamily Tenebrioninae and *Zophosis* (*Onychosis*) *gracilipes* (Lockey, 1984b), subfamily Tentyriinae. Trimethylalkanes were only tentatively identified in *Z.* (*Onychosis*) *gracilipes*, where they comprise a mixture of the 4,8,12-trimethylisomers of C_{24} and C_{26}. In *C. laevioctostriatus* and *P. gibbus* trimethylalkanes occur as mixtures of mainly 9,13,17- to 13,17,21-trimethylisomers with chain length C_{31}–C_{35} (*C. laevioctostriatus*) and C_{29}–C_{35} (*P. gibbus*). These mixtures clearly differ from the simpler mixture of *O. boschimana* and from that of *O. bicolor* which includes trimethylalkanes with variable branching patterns (Tables 1 and 2).

It is only recently that tetramethylalkanes have been identified with certainty in insect hydrocarbon mixtures (Nelson *et al.*, 1988). In some earlier work, Dubis and co-workers (1986) identified the 3,10,16,23-tetramethylisomers of C_{31} and C_{33} in the hydrocarbon mixture of the Colorado beetle, *Leptinotarsa decemlineata* but their identification is doubtful for the following reasons (a) the retention indices of the C_{31} and C_{33} tetramethylalkanes are about 120 retention units too high, (b) a 3,10,16,23-tetramethylalkane has six methylene groups separating branches 1 and 2 and branches 3 and 4. Insects synthesise their methylalkanes via the elongation-decarboxylation pathway and as a result branches are separated by an odd number of methylene groups, one group coming from a methylmalonyl derivative, the others from a 2-carbon malonyl derivative (Blomquist and Dillwith, 1985) and (c) the fragmentation pattern of 3,10,16,23-tetramethyltritriacontane should comprise ion doublets at m/z 56/7 (C_4) and m/z 168/9 (C_{12}) and enhanced fragment ions at m/z 169 (C_{12}), m/z 267 (C_{19}), m/z 281 (C_{20}), m/z 379 (C_{27}) and m/z 491 (C_{35}). The mass spectrum given by Dubis and co-workers (their Fig. 5) for this component shows four extraneous ion doublets at m/z 252/3 (C_{18}), m/z 266/7 (C_{19}), m/z 280/1 (C_{20}) and m/z 378/9 (C_{27}) and only three of the expected fragment ions.

The tetramethylalkanes identified by Nelson and co-workers (1988) in the tsetse fly, *Glossina* occur as mixtures of the 3,7,11,15- to 11,15,19,23-tetramethylisomers of C_{30}–C_{37}. Except for the 8,12,18,22-tetramethylisomer of C_{36}, most of the tetramethylalkanes have their branches separated by three methylene groups. The 8,12,18,22-tetramethylisomer of *Glossina* is similar to those tentatively identified in *O. bicolor*, except that five methylene groups separate branches 2 and 3 in the latter isomer and branches 1 and 2 in the isomers of *O. bicolor* (Table 1).

After determining the hydrocarbon compositions of *Onymacris plana*, *O. rugatipennis* (Lockey, 1982a) and *O. marginipennis* (Lockey, 1982b), genus *Onymacris* was provisionally characterized by the presence of $n$$C_{31}$ and high proportions of the 3-methylisomers of C_{27} and C_{29}. *O. bicolor* and *O. boschimana* with a high proportion of 3-methylheptacosane and 3-methylnonacosane, respectively, show only one of these characteristics and the difficulty remains of characterizing the genus and separating it unambiguously from other closely related genera such as *Physadesmia* (Lockey, 1982c) and *Metriopus* (Lockey, 1984a). Part of the difficulty probably derives from subdivisions within genus *Onymacris* and from the close relationship of the three genera (Penrith, 1975, 1984).

In these previous investigations, qualitative and quantitative differences in hydrocarbon composition

clearly separated *O. marginipennis* from *O. plana* and *O. rugatipennis*, while the latter two species were distinguished by quantitative differences in composition. This separation agreed with the then division of *Onymacris* into three groups, with *O. marginipennis* in group 1 and the other two species in different subgroups of group 3 (Penrith, 1975). In more recent work, Penrith (1984) divides the 14 species of the genus into two groups, the *O. multistriata–O. lobicollis* group, which includes *O. boschimana* and *O. rugatipennis* (Lockey, 1982a) and the *O. hottentota–O. bicolor* group which includes *O. plana* (Lockey, 1982a) and *O. marginipennis* (Lockey, 1982b). In the cladogram given by Penrith (1984) the five examined species of *Onymacris* are placed in the following order: group 1; *O. boschimana* (OBS), *O. rugatipennis* (OR): group 2; *O. plana* (OP), *O. marginipennis* (OM) and *O. bicolor* (OBC). The cladistic analysis undertaken by Penrith (1984) indicates further that within group 1, *O. boschimana* and *O. rugatipennis* have separate lineages and that within group 2, *O. plana* has a separate lineage while *O. bicolor* and *O. marginipennis* are closely related. The latter two species belong to a subgroup of five "white" *Onymacris* species which have lost the black coloration of their elytra and aedeagus and which show some interspecific hybridization (Penrith, 1975).

In a recent multivariate analysis of compositional data (Lockey and Metcalfe, 1988), *O. plana* (OP) is placed closer to *O. marginipennis* (OM) (both group 2) than to *O. rugatipennis* (OR) (group 1) which supports division of the five examined species of genus *Onymacris* into two rather than three groups. Further, group 1 species tend to have fewer hydrocarbons with a narrower chain length range than group 2 species and their hydrocarbon mixtures tend to contain a few components in high abundance rather than several components in moderate abundance which occurs in group 2 species. The five species show a transition in this tendency from *O. boschimana* (OBS) of group 1 to *O. bicolor* (OBC) of group 2. Thus, *O. boschimana* has a mixture which comprises 77 hydrocarbons ranging in chain length from C_{20} to C_{31} and which contains five components in high abundance accounting for nearly 80% of the mixture. *O. bicolor* by contrast, has a mixture which consists of 124 hydrocarbons ranging in chain length from C_{19} to C_{38} and which contains eleven moderately abundant components accounting for approximately 77% of the mixture. *O. plana* (group 2) occupies an intermediate position in this transition in that it has a mixture which consists of 81 hydrocarbon ranging in chain length from C_{23} to C_{33} and which contains six components in moderately high proportions accounting for approximately 64% of the mixture. The tendency for chain length range to increase in the five species is most marked in the dimethylalkanes which show the following sequence: Group 1, OBS (C_{25}–C_{31}), OR (C_{27}–C_{35}); group 2, OP (C_{25}–C_{33}), OM (C_{25}–C_{37}) and OBC (C_{21}–C_{37}). These species appear in the same sequence in cladistic analysis (Penrith, 1986).

Division of the five examined species into two groups is also supported to some extent by the quantitative characteristics of their mixtures of internally branched monomethylalkanes. Whereas all five species have high proportions of the monomethylisomers of C_{27} and C_{29}, *O. boschimana* and *O. rugatipennis* (group 1) have a higher proportion of nonacosanes than the three species of group 2. The latter in turn have higher proportions of the monomethylisomers of C_{26} and the 7- to 13-methylisomers of C_{27}.

As with monomethylalkanes, all five species have high proportions of the dimethylisomers of C_{27} and C_{29}. However, the dimethylalkane mixture of OP (group 2) shares more characters with those of OBS and OR (group 1) than with OBC and OM. Thus, OP, OBS and OR have a higher proportion of the dimethylisomers of C_{29} and C_{30} than OM and OBC, while OP and OR alone have high proportions of the dimethylisomers of C_{31}, C_{32} and C_{33}. At the same time, OM and OBC have a higher proportion of the dimethylisomers of C_{26} and C_{27} than the other three species.

Apart from the presence of trimethyl- and tetramethylalkanes, the hydrocarbon mixture of *O. bicolor* is qualitatively and quantitatively very similar to that of *O. marginipennis* (Lockey, 1982b) and these similarities reflect the close relationship between the five "white" *Onymacris* species (Penrith, 1975). Both species have mixtures with a wide chain length range (OBC, C_{19}–C_{38}; OM, C_{23}–C_{37}) and the same or similar components in enhanced abundance. The most abundant components shared by the two mixtures are: (1) class A: $nC_{27} > nC_{29}$ (OBC and OM), (2) class C2: the 3-methylisomer of C_{27} (OBC), the 3-methylisomer of $C_{27} > C_{29}$ (OM) and (3) class D, the monomethylisomers of $C_{27'} > C_{29'} > C_{25} > C_{28}$ (OBC), $C_{29'} > C_{27'} > C_{28'} > C_{30}$ (OM). The two species also have high proportions of terminally branched (type 2) dimethylalkanes which account for about 73–74% of their dimethylalkane mixtures. Only *Stenocara gracilipes* (Lockey, 1982c) among the nine examined adesmiine species has the same proportion of type 2 dimethylalkanes. Comparable values for other examined species of genus, *Onymacris* are: *O. plana* (14%), *O. rugatipennis* (31%) (Lockey, 1982a) and for the remaining examined genera of tribe Adesmiini, *Physadesmia globosa* (19%) (Lockey, 1982c), *Metriopus depressus* (21%) and *Renatiella scrobipennis* (68%) (Lockey, 1984a). The type 2 dimethylalkane mixtures of *O. bicolor* and *O. marginipennis* include 6,*x*-dimethylisomers (OBC, 6,10–C_{24}; OM, 6,14–C_{30} and 6,16–C_{26}) which other examined adesmiine species lack and an extensive series of 7,*x*-dimethylisomers, where *x* is an odd number ranging from 11 to 17. The latter series accounts for about 49% of the dimethylalkanes of *O. bicolor* and ranges in chain length from C_{21} to C_{37} while in *O. marginipennis* the series accounts for about 14% of the dimethylalkane mixture and ranges from C_{25} to C_{37}. Both species have 8 of the 7,*x*-dimethylisomers. Comparable values for the other examined adesmiine species range from zero (*O. plana* and *M. depressus*) to three (*P. globosa*).

Insects synthesize their hydrocarbons via an elongation-decarboxylation pathway (ED pathway) in which 2-carbon malonyl derivatives are incorporated to form an even chain fatty acid, which on decarboxylation yields a hydrocarbon with one less

carbon. In the biosynthesis of methylalkanes, malonyl derivative is replaced by methylmalonyl derivative (the branch donor) which is incorporated in the early stages rather than the late stages of chain elongation (Blomquist and Dillwith, 1985; Dwyer *et al.*, 1981a). Most methylalkanes are synthesized via the malonyl/methylmalonyl (ED) pathway and as a result they have odd chains and their branches positioned at an odd-numbered carbon (odd/odd methylalkanes). Methylalkanes with even chains and with their branches positioned at either an odd- or an even-numbered carbon also occur in insect hydrocarbon mixtures though in much lower proportions (Table 3) (Lockey, 1988). The biosynthetic pathway for even-chain methylalkanes probably includes the incorporation of a 3-carbon derivative of some type into the ED pathway either before the first methylmalonyl derivative is incorporated, for those methylalkanes with their branches positioned at even-numbered carbons (even/even methylalkanes) or after incorporation of the last methylmalonyl derivative, for those with their branches at odd-numbered carbons (even/odd methylalkanes). Odd chain methylalkanes with their branches positioned at even-numbered carbons (odd/even methylalkanes) occur only very rarely, if at all (Lockey and Oraha, 1990). Biosynthesis of these latter methylalkanes via an ED pathway requires the incorporation of 2,3-carbon derivatives; one before incorporation of the first methylmalonyl derivative, the other after incorporation of the last methylmalonyl derivative (Lockey, 1988).

Insects often have complex isomeric mixtures of methylalkanes. *O. bicolor*, for example has 121 methylalkanes, including 57 monomethylalkanes and 41 dimethylalkanes. The biosynthetic pathways which *O. bicolor* may use to synthesize its complex mixture of dimethylalkanes are indicated in Table 3 which gives the likely incorporation sequence of derivatives during chain elongation up to decarboxylation. The suggested incorporation sequence of derivatives for 3,13-C_{23} (odd/odd dimethylalkane), for

Table 3. The likely incorporation sequence of derivatives up to decarboxylation for the type 1 and 2 dimethylalkanes of *O. bicolor*

Isomer	CL	Incorporation sequence							Approx. % comp.
Type 2 dimethylalkanes									
313	C_{23}		M	MM	4M	MM	5M		0.1
37	C_{25}		M	MM	M	MM	9M		0.4
37	C_{26}		M	MM	M	MM	8M	3C	t
39			M	MM	2M	MM	7M	3C	
37	C_{27}		M	MM	M	MM	10M		1.0
39			M	MM	2M	MM	9M		
315	C_{29}		M	MM	5M	MM	7M		0.3
414	C_{24}	3C		MM	4M	MM	5M		t
48	C_{26}	3C		MM	M	MM	9M		0.2
48	C_{28}	3C		MM	M	MM	10M		0.1
410		3C		MM	2M	MM	9M		
412		3C		MM	3M	MM	8M		
59	C_{23}		2M	MM	M	MM	7M		0.3
515	C_{25}		2M	MM	4M	MM	5M		0.2
59	C_{26}		2M	MM	M	MM	7M	3C	0.1
59	C_{27}		2M	MM	M	MM	9M		1.0
59	C_{28}		2M	MM	M	MM	8M	3C	1.2
511			2M	MM	2M	MM	7M	3C	
513			2M	MM	3M	MM	6M	3C	
59	C_{29}		2M	MM	M	MM	10M		0.2
610	C_{26}	3C	M	MM	M	MM	7M		t
711	C_{21}		3M	MM	M	MM	5M		0.1
713	C_{23}		3M	MM	2M	MM	5M		0.4*
711	C_{24}		3M	MM	M	MM	5M	3C	0.1
715	C_{25}		3M	MM	3M	MM	5M		0.9*
717			3M	MM	4M	MM	4M		
717	C_{29}		3M	MM	4M	MM	6M		7.9
715	C_{37}		3M	MM	3M	MM	11M		0.1
717			3M	MM	4M	MM	10M		
Type 1 dimethylalkanes									
913	C_{23}		4M	MM	M	MM	5M		0.7
913	C_{29}		4M	MM	M	MM	8M		0.5*
913	C_{32}		4M	MM	M	MM	8M	3C	t
1014	C_{24}	3C	3M	MM	M	MM	5M		0.1
1014	C_{26}	3C	3M	MM	M	MM	6M		0.1*
1115	C_{25}		5M	MM	M	MM	5M		0.7
1115	C_{26}		5M	MM	M	MM	4M	3C	0.1*
1115	C_{27}		5M	MM	M	MM	6M		1.8
1115	C_{29}		5M	MM	M	MM	7M		0.5*
1115	C_{30}		5M	MM	M	MM	6M	3C	0.1*
1317	C_{29}		6M	MM	M	MM	6M		0.5*
1317	C_{30}		6M	MM	M	MM	5M	3C	0.1*

*Estimate. Approximate percentages: type 1, 5.2%, type 2, 14.6%. Dimethylalkanes: odd/odd, approx. 17.6% (88.9%); even/odd, approx. 1.7% (8.6%); even/even, approx. 0.5% (2.5%).

example, is: 1, malonyl (M), 1, methylmalonyl (MM), 4, malonyl (4M), 1, methylmalonyl (MM) and 5, malonyl (5M). This results in the synthesis of a C_{24} branched fatty acid, which on decarboxylation yields a C_{23} dimethylalkane.

It is clear from Table 3 that minor changes to the biosynthetic pathway, perhaps brought about by slight alterations in enzyme concentration, can affect branch position and chain length. For example, branch position and chain length in odd/odd dimethylalkanes can be changed by incorporating a different number of malonyl derivatives into the chain before the first methylmalonyl derivative and after the second methylmalonyl derivative, respectively. The same applies to even-chain dimethylalkanes, though the presence of a 3-carbon derivative will affect branch positions and chain length.

It is also possible to see from Table 3 that in certain cases, slight changes to a biosynthetic pathway can result in the biosynthesis of either a monomethyl-, dimethyl- or trimethylalkane. For example, the hydrocarbon mixture of *O. bicolor* (Table 1) contains the following series of methylalkanes: 5-, 5,9- and 5,9,13-C_{23}; 6-, 6,10- and 6,10,14-C_{24} and 7-, 7,15- and 7,17- and 7,11,15-C_{25}. The incorporation sequence up to decarboxylation for the first series is: 5-C_{23}, 2M MM 9M; 5,9-C_{23}, 2M MM M MM 7M and 5,9,13-C_{23}, 2M MM M MM M MM 5M. Clearly, the first three steps in the incorporation sequence of the monomethyl-, dimethyl- and trimethylalkane are the same. Thereafter, the incorporation of additional methylmalonyl derivatives during chain elongation determines whether a dimethyl- or trimethylalkane is synthesized.

Table 3 also highlights the differences in *O. bicolor* between type 1 dimethylalkanes which have their branches centrally positioned and separated exclusively by three methylene groups and type 2 dimethylalkanes which have their first branch positioned at carbon 3–7 and separated from the second branch by an odd and variable number of methylene groups. In the biosynthesis of the two types of dimethylalkanes, it appears that when incorporation of the first methylmalonyl derivative into the chain is early it may be followed by the incorporation of several additional malonyl derivatives before the second methylmalonyl derivative is incorporated whereas when incorporation is delayed only one malonyl derivative is incorporated into the chain before incorporation of the second methylmalonyl derivative. The differences between type 1 and 2 dimethylalkanes are quite marked in most examined tenebrionid species (Lockey, 1988) and this suggests that two distinct types of ED pathway may be used in their biosynthesis. It may be that in this group, type 2 dimethylalkanes with their mainly terminal branches are synthesized via an ED pathway which involves the elongation of a long-chain fatty acid, as in the biosynthesis of olefins and n-alkanes (Blomquist and Dillwith, 1985; Blomquist *et al.*, 1987; Dwyer *et al.*, 1981b; Vaz *et al.*, 1988) rather than a pathway which involves *de novo* synthesis. Clearly the biosynthetic pathways for type 2 dimethylalkanes are more varied than those for type 1 dimethylalkanes. Indeed, for the odd/odd type 1 dimethylalkanes of *O. bicolor* (Table 3) the first 7–9 steps in chain elongation are very similar, differences being confined to the number of malonyl derivatives incorporated into the chain after the second methylmalonyl derivative. This may explain why type 1 rather than type 2 dimethylalkanes are more abundant in most insect hydrocarbon mixtures (Lockey, 1988). However, in some groups, such as acridid orthopterans, it is the type 1 dimethylalkanes which have variable branching patterns while type 2 dimethylalkanes are often absent (Lockey and Oraha, 1990).

In some earlier work (Lockey, 1988; Lockey and Oraha, 1990), it was suggested that the main function of the hydrocarbon mixture of an insect's cuticular lipid is to provide a fluid matrix for the polar components and that hydrocarbon composition is related to environment as it determines fluidity and the range of temperatures over which optimum fluidity occurs.

The hydrocarbon mixture of *O. boschimana* has a chain length range of 11 carbons and a mean chain length equal to 29 carbons. Further, the mixture contains five high abundance components accounting for about 80% of the mixture which gives an average value of 16% for abundant components. By contrast, the hydrocarbon mixture of *O. bicolor* has a chain length range of 19 carbons, a mean chain length equal to 27.4 carbons and an average value of 7.0% for abundant components. From these values, it seems likely that the two hydrocarbon mixtures will have different melting characteristics with the mixture of *O. boschimana* melting over a higher and narrower range of temperatures than the mixture of *O. bicolor*.

According to Penrith (1975), *O. boschimana* inhabits the inland sandy plains of the Namib desert, whereas *O. bicolor* inhabits the vegetationless white coastal dunes. Coastal temperatures of the Namib desert are lower than those of the interior. For example, annual coastal temperatures at Swakopmund range from 8 to 23.4°C, with an average annual temperature of 16.8°C (Wellington, 1967), while a few miles inland at Gobabeb annual temperatures range from 26.6 to 33.3°C, with an annual average of 30°C (Seely and Stuart, 1976). From this it appears that both species show a relationship between the melting range of their hydrocarbon mixtures and the range of environmental temperatures they are likely to encounter. *O. boschimana* has a hydrocarbon matrix with a fluidity suited to the higher and narrower range of temperatures likely to occur in the inland habitat whereas *O. bicolor* has a matrix fluidity suited to the wider and lower range of temperatures obtaining in the coastal habitat.

Two species of *Lepidochora* (Lockey, 1985) show a similar relationship. *L. discoidalis* (LD) which inhabits the inland dunes of the Namib desert has a hydrocarbon mixture with a higher mean chain length and a higher average value for abundant components than *L. eberlanzi* (LE), which inhabits coastal dunes (Koch, 1962). Although *L. eberlanzi* has a smaller chain length range than *L. discoidalis* (LE 10 carbons; LD, 12 carbons), the latter has a much simpler hydrocarbon mixture (LD, 26 hydrocarbons; LE, 66 hydrocarbons), nearly 74% of which consists of nC_{33} which melts at 71.8°C. It follows that the coastal species, *L. eberlanzi* will have a hydrocarbon mixture with a lower and wider melting range

than the inland species *L. discoidalis* because of its more complex composition and its lower mean chain length.

Of the other examined species of *Onymacris*, *O. rugatipennis* which inhabits dry river beds with luxuriant vegetation (Penrith, 1975) has a hydrocarbon mixture with the characteristics of an inland species and similar to those of *O. boschimana*. *O. plana*, however, which lives on wind-blown dunes in the vicinity of plants, combines the inland characteristics of a narrow chain length range and a high mean chain length with the coastal characteristics of a low average value for abundant components. *O. marginipennis* which inhabits hummocks of *Salsola* sp. and other succulent plants, occurs on the coast and inland along dry river beds. Its hydrocarbon mixture has the same characteristics as those of *O. bicolor*, though to a less marked extent.

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