CHEMICAL COMPOSITION OF COMPOUNDS PRODUCED BY THE PEA APHID *Acyrthosiphon pisum* (HARRIS): PENTANE AND CHLOROFORM–METHANOLIC EXTRACTS OF BODY LIPIDS

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The pentane (B) and chloroform–methanolic (C) extract of crushed bodies of the pea aphid *Acyrthosiphon pisum* (HARRIS) was analysed. Homologous series of alkanes (inclusive of *trans*-β-farnesene), higher esters, saturated and unsaturated triglycerides, free aliphatic acids and alcohols, 1,3- and 1,2-diglycerides and ethanediol monoesters were detected by means of chromatographical and spectrometrical methods. The results were compared with those obtained by analysis of the pentane extract (A) of surface lipids from intact bodies.

The pentane extract (A) of surface lipids from intact pea aphid *Acyrthosiphon pisum* (HARRIS) was analysed in the previous paper[1]. The insect bodies were then crushed and extracted with pentane again (extract B) and chloroform–methanol (extract C). Analysis of extracts B and C is the object of the present paper.

EXPERIMENTAL

For the material, extraction, and chromatography of extracts on a column of silica gel see the previous paper[1]. The first column chromatography was efficient enough to separate the free acids and alcohols. Compounds of Table I are arranged accordding to the decreasing R_F values on thin-layer chromatography on silica gel in hexane–ether as eluent. Esterification of free acids, transesterification of higher esters and glycerides, silylation of free alcohols, gas chromatography, infrared spectroscopy and mass spectrometry were performed as described previously[1].

RESULTS AND DISCUSSION

Analysis of extracts B and C is shown in Table I along with the earlier analytical data of extract A. The substantial portion of hydrocarbons in all the three extracts is formed on principle by two homologous series (I and II) of straight and branched alkanes of the $C_{16}-C_{38}$ range. Thus, the content of branched hydrocarbons is almost 20% in extract A, 62·5% in extract B, and 71·7% in extract C (Table II). The hydrocarbon mixtures are rather complex as inferred from chromatograms

TABLE I

Group Composition of Extracts A, B, and C

Spot No	Compound[a]	A (684·0 mg)		B (1 912·0 mg)		C (3 329·0 mg)	
		yield mg	% E[b]	yield mg	% E	yield mg	% E
1	alkanes	27·3	4·2	15·0	0·80	47·6	2.8
2	—	+[c]	+	+	+	—	—
3	*trans*-β-farnesene	+	+	0·8	0·04	1·0	0·06
4	—	+	+	+	+	+	+
5	—	+	+	+	+	+	+
6	—	—	—	+	+	+	+
7	esters	17·1	2·7	7·2	0·39	22·0	1·3
8	methyl esters	—	—	—	—	32·6	1·9
9	aldehydes	55·5	8·6	1·0	0·05	+	+
10	—	1·4	0·22	2·3	0·12	—	—
11	—	—	—	4·3	0·23	—	—
12	—	—	—	6·8	0·36	2·1	0·12
13	saturated triglycerides	91·8	14·2	1 265·9	67·7	876·9	51·7
14	unsaturated triglycerides	83·3	12·9	131·0	7·0	85·0	5·0
15	—	1·0	0·15	—	—	6·0	0·35
16	—	1·0	0·15	—	—	—	—
17	fatty acids	270·0	41·8	400·7	21·4	524·0	30·9
18	—	23·1	3·6	—	—	—	—
19	alcohols	60·5	9·4	+	+	16·0	0·94
20	—	—	—	—	—	22·4	1·3
21	diglycerides		—	35·0	1·9	22·0	1·3
22	ethanediol monoesters	—	—	—	—	26·4	1·6
23	α-hydroxy acids	13·3	2·1	—	—	13·4	0·79
	not eluted	38·7	—	42·0	—	1 631·6	—

[a] So far as identified; [b] % in the column effluent; [c] the symbol + designates traces.

TABLE II

Composition (%) of Alkanes from Extracts A, B and C

Alkanes	A	B	C
Normal	80·3	37·5	28·3
Branched series I	9·3	25·6	30·7
series II	8·9	36·9	41·0
series III	1·2	—	—
series IV	0·3	—	—

obtained on packed columns. By means of gas chromatography-mass spectrometry (GLC–MS), the sesquiterpene *trans*-β-farnesene was identified in all the three extracts A, B, and C. From extracts B and C, only about 1 mg of this substance was isolated in pure state. However, as suggested by thin-layer chromatography of original extracts, its actual amount in insect bodies should be approximately ten times higher. *trans*-β-Farnesene, excreted from cornicles of pea aphids and acting as alarm pheromon, has also been detected in other aphid species[2-6]. This sesquiterpene has also been identified by mass spectra; a gaseous sample obtained by the head space method[7] from crushed aphid bodies was injected by means of a modified syringe[8] into a combined GLC–MS apparatus.

The content of particular ester homologues in extracts B and C is almost the same. When compared with extract A, the maxima of C_{44} and C_{46} homologues are not as significant in extracts B and C. Transesterification of esters of extracts B and C afforded results similar to those obtained in the case of extract A (ref.[1]). In addition to a group of higher esters, some methyl esters (the esterified $C_{18:2}$, $C_{18:1}$, $C_{16:1}$, and $C_{14:0}$ acids predominated) were isolated and identified (IR: 1744, 1200, and 1173 cm^{-1} (—COOR), 1438 cm^{-1} (—$COOCH_3$), and 3085, 3015, and 1654 cm^{-1} (>C=C<)) in extract C. To our opinion, these methyl esters are artefacts produced from about 1% of the free acids by the action of the chloroform–methanol extracting agent.

No aldehydes were found in extracts B and C in contrast to the surprisingly high content of aldehydes (8·6%) in extract A. The aldehydes are thus contained in surface lipids of the pea aphid only, similar to free alcohols. The occurence of aliphatic aldehydes in insect lipids is rare[9].

The ratio of saturated triglyceride homologues in extracts B and C and their chemical composition is almost identical with that observed in extract A. On the other hand, the amount of these glycerides is many times higher in extracts B and C. The principal component of all the three extracts A, B, and C (its content is the highest in extract A) is represented by a triglyceride containing myristic acid at positions 1 and 3 and sorbic acid at position 2. Mass spectra obtained by measurements of extract C by the direct inlet at various temperatures (170–270°C) were used to determine molecular weights of all important components of this mixture and to detect most acids contained in triglycerides. The RCO^+, $(RCO + 74)^+$, and $(RCO + 128)^+$ ions served for determination of the present acids[10,11] except for those unsaturated acids, all the carbon atoms of which are incorporated in the system of conjugated double bonds (such as with the $C_{6:2}$, $C_{8:3}$, and other acids). The mass spectra of triglycerides containing these acids are characterised by unusually intensive peaks of R_cCO^+ ions (wherein R_c designates the acid residue containing the system of conjugated double bonds) while the corresponding $(R_cCO + 74)^+$ or $(R_cCO + 128)^+$ ions are almost absent as it has been observed earlier[1] in the case of the C_{14}—$C_{6:2}$—C_{14} triglyceride. The molecular ions of triglycerides were also detectable in the mixed spectrum

by means of the corresponding $(M - 18)^{+\bullet}$ ions. In this manner, the presence of the following triglycerides was established: $C_{32:2}$ (C_{12}—$C_{6:2}$—C_{14}, M.w. 578), $C_{34:3}$ (C_{12}—$C_{8:3}$—C_{14}, M.w. 604), $C_{34:2}$ (C_{14}—$C_{6:2}$—C_{14}, M.w. 606; the main component), $C_{36:3}$ (C_{14}—$C_{8:3}$—C_{14}, M.w. 632), $C_{38:3}$ (C_{14}—$C_{14:3}$—C_{14}, M.w. 660), $C_{40:4}$ (C_{14}—$C_{16:4}$—C_{14}, M.w. 686), and $C_{48:5}$ (C_{14}—$C_{20:5}$—C_{14}, M.w. 796). The character of additional substances present of molecular masses 1000 and 1026 was not determined. Our results concerning composition of saturated as well as unsaturated triglycerides are in accordance with those reported in some recent papers[12-15] on the basis of which myristic acid ($C_{14:0}$), hexanoic acid ($C_{6:0}$), and sorbic acid ($C_{6:2}$) may be regarded as the main acid components of aphid triglycerides.

Similar to extract A, the content of free acids in extracts B and C is surprisingly high. The acids $C_{18:2}$, $C_{18:1}$, $C_{18:0}$, $C_{14:0}$, and $C_{16:1}$ again predominate. In view of the ready decomposition of unsaturated acids it may be assumed that a portion of acids polymerised during isolation and identification of other substances. The original amount of free acids might be probably somewhat higher.

In contrast to extract A, extract C contained only small amounts of free alcohols (*cf.* the aldehydes). The extent of the homologous series and the ratio of homologues is in extracts A and C very similar (Table I).

Diglycerides were found in extracts B and C only (IR: 1745 and 1167 cm^{-1} (—COOR), 3625 and 1050 cm^{-1} (—OH), and 3085 and 3015 cm^{-1} (>C=C<)). In both cases, two isomeric series of homologues were detected by gas chromatography in the range (overall number of carbon atoms) from C_{21} (C_{12} and C_6 acids) to C_{39} (C_{30} and C_6 acids), the C_{23} homologue (C_{14} and C_6 acids) being predominating (about 90%). The peaks of the two homologous series were almost overlapping; peaks of series II (longer retention times) predominated about ten times over peaks of the somewhat more volatile isomers of series I. After transesterification of both the diglyceride series, the $C_{6:0}$ acid was present along with a series of even homologous acids from $C_{12:0}$ to $C_{30:0}$ (the $C_{14:0}$ acid predominated), as shown by gas chromatography. The presence of small amounts of the $C_{16:1}$ and $C_{18:1}$ acids may also be inferred from these chromatograms.

Since mass spectra of diglycerides do not make possible an unequivocal determination of positions of the particular acids[16], the diglycerides of extract C were subjected to trimethylsilylation and the resulting mixture was analysed by GC–MS. In the record of the total ion current, both the homologous series of trimethylsilylated diglycerides were resolved; it was therefore possible to obtain mass spectra of members of both the isomeric series (Table III). As shown by Barber and coworkers[16], the mass spectra of trimethylsilylated 1,3- and 1,2-diglycerides can be readily resolved by means of $[-MCH_2OCOR]^+$ and $[M-\{R{=}C{=}O + Si(CH_3)_3\}]^+$ ions. The former ion type is exclusively present in mass spectra of 1,3-derivatives while the latter is significant in spectra of 1,2-derivatives only. Accordingly, the mass spectrum (fairly

intensive *f* and *g* ions and very low peaks of *h* and *i* ions) of the series I C_{23} diglyceride trimethylsilyl derivative corresponds to the 1,2-isomer. The other three spectra of the series II C_{21}, C_{23}, and C_{25} homologues (very intensive *h* and *i* ions and negligible peaks of *f* and *g* ions) point to 1,3-diglyceride derivatives as the predominant component of this trimethylsilylated fraction of extract C.

A small amount of ethanediol monoesters was detected in extract C only (IR: 1742 and 1173 cm^{-1} (—COOR), 3630 cm^{-1} (—OH), and 3090, 3015, and 1640 cm^{-1} (>C=C<)). Two homologous series I and II could be detected by gas chromatography. Series I contains ethanediol monoesters of unsaturated $C_{16:1}$ (3·8%) and $C_{18:1}$ (40·9%) acids while ethanediol monoesters of saturated $C_{12:0}$ (0·6%), $C_{14:0}$ (11·3%), $C_{16:0}$ (4·7%), $C_{18:0}$ (29·8%), and $C_{20:0}$ (6·2%) acids are present in series II. The same results were also inferred from gas chromatography of methyl esters obtained by transesterification of the ethanediol monoester mixture. A combined GC–MS method was used to determine the mass spectra of main components of the original fraction. The observed intensive peaks of *m/e* 61, 98, 104, 112, 117, and M−61 ions are considered[17] as characteristic of ethanediol monoesters. For a survey of ethanediol monoesters hitherto isolated from plants, animals, and microorganisms see the paper of Bergelson[18].

The constitution of α-hydroxy acids isolated from extract A has been reported earlier[1]. A small amount of substances of the same R_F values on a thin layer of silica gel was also isolated from extract C but the identification failed because of their instability despite the storage in a refrigerator.

TABLE III

Values of *m/e* and Relative Intensities (in % of base peak) of *a*−*i* ions in Mass Spectra of Diglyceride Trimethylsilyl Ethers (C_{21}, C_{23}, and C_{25} homologues in the series II and the C_{23} homologue in the series I) from Extract C

Ion	Ion structure[a]	C_{21} (II)		C_{23} (I)		C_{23} (II)		C_{25} (II)	
		m/e	%	*m/e*	%	*m/e*	%	*m/e*	%
a	M − 15	429	11	457	12·2	457	17	485	14
b	R^1CO	99	100	99	73	99	100	99	100
c	$R^1CO + 74$	173	27	173	61	173	29	173	28
d	R^2CO	183	40	211	21·6	211	29	239	18
e	$R^2CO + 74$	257	15	285	37·3	285	13	313	20
f	$M - \{R^1{=}C{=}O + Si(CH_3)_3\}$	273	—	301	10·2	301	—	329	—
g	$M - \{R^2{=}C{=}O + Si(CH_3)_3\}$	189	5	189	14·3	189	5·6	189	—
h	$M - CH_2OCOR^1$	315	45	343	—	343	53	371	31
i	$M - CH_2OCOR^2$	231	62	231	—	231	71	231	56

[a] $R^1 = C_5H_{11}$—; $R^2 = C_nH_{2n+1}$— (n = 11, 13, and 15).

The following conclusion can be drawn from comparison of extracts A, B, and C. The amount of substances obtained by the pentane extraction of crushed pea aphids (extract B) is about three times higher than the amount of surface lipids obtained by extraction of intact insect bodies with the same solvent (extract A). In addition to lipids, some more polar substances (49%) were extracted with the chloroform–methanol solvent mixture (extract C); when subjected to column chromatography, the polar substances remained adsorbed to silica gel. The crushed pea aphids containing 80% of water[1] cannot thus be exhaustively extracted with pentane alone, as shown by quantitative evaluations of extracts B and C; the use of the chloroform–methanol solvent mixture was therefore fully justified[19]. The extracts A, B, and C differ in their chemical composition particularly in the case of alkanes, aldehydes, free alcohols, diglycerides, ethanediol monoesters, and hydroxy acids. Except for alkane fractions, the content of extracts B and C is similar.

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