CHEMICAL COMPOSITION OF COMPOUNDS PRODUCED BY THE PEA APHID *Acyrthosiphon pisum* (HARRIS): PENTANE EXTRACT OF SURFACE LIPIDS

K.STRÁNSKÝ[a], K.UBIK[a], J.HOLMAN[b] and M.STREIBL[a]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6,*
[b] *Institute of Entomology, Czechoslovak Academy of Sciences, Prague 2*

Received May 4th, 1972

Surface lipids of the pea aphid *Acyrthosiphon pisum* were analyzed. Using chromatographic and spectroscopic methods, normal and branched alkanes ($C_{16}-C_{39}$), simple alkyl esters ($C_{32}-C_{54}$), aldehydes ($C_{22}-C_{34}$), saturated and unsaturated triglycerides (mostly C_{37}), free saturated and unsaturated acids ($C_{12}-C_{24}$), free primary alcohols ($C_{20}-C_{32}$) and free α-hydroxy acids (C_9-C_{21}) were found.

Knowledge of the lipid substances of insects helps in elucidating the problems of metabolism, composition and action of pheromones and, finally, it may serve in the fight against insect pests. Indirect evidence exists that resistance to insecticides in some insect groups[1] is associated with the lipid metabolism. Some fatty acids mimic the effect of the juvenile hormone and can thus affect the insect metamorphosis[2].

Acyrthosiphon pisum (HARRIS) (*Homoptera, Aphididae*) is an important pest of some leguminous cultures. In Europe, there are regular outbreaks of the aphid on alfalfa. A clone introduced to North America is particularly noxious to pea. The population of the species is composed of several more or less defined races which differ mainly in host preference and in colour. From the point of view of lipid content the present species has not yet been examined. For analysis, the surface of intact individuals was first extracted with pentane (extract A), the extracted material was then ground, extracted again with pentane (extract B) and finally with a mixture of chloroform–methanol (extract C). The results of analysis of extract A are presented in this communication.

EXPERIMENTAL

Materials and Methods

Animals. For the analysis, a laboratory culture of *Acyrthosiphon pisum* (HARRIS, 1776) from the Institute of Entomology, Czechoslovak Academy of Sciences, was used. The material belongs

to the alfalfa race of the species. The aphids were reared at 17—18°C on a 18 h day regime on young broad bean plants cultivated in spruce and pine sawdust. When taking the samples, a small sample was always removed for an analysis of the number and weight of the various instars (Table I). The dry weight (dried at 60°C) represents 19% of live weight. Table I shows that the sample contained different instars of parthenogenetic females. More than 70% total live weight was represented by instars III—IV and by imagoes of apterous females, 15% by alate females and some 12% by the youngest instars where the future form (apterous or alate) was not yet defined. In addition, the sample contained a smaller amount of exuviae, the weight of which was negligible.

Extraction. A: 156 g live aphids were submerged in n-pentane. After 4 h of standing the suspension was poured into a column and continually extracted at room temperature with n-pentane (the animals represent some 370 ml). 250 ml fractions were collected. After the 6th fraction had been taken, the material was left in the column overnight and fraction 7 (which did not differ from fractions 1—6 on thin-layer chromatography) was collected on the following day. A total of 684 mg extract A was obtained. B: Material after extraction A was frozen with liquid nitrogen, triturated in a porcelain mortar and extracted in a separating funnel with 75 ml n-pentane. Twelve portions yielded 1·91 g extract B. C: Material after extraction B was extracted in the same way (see extraction B) with a mixture of chloroform–methanol (17 : 3). 32 extract portions yielded 3·32 g extract C.

Chromatography. 684 mg extract A was adsorbed on 3·5 g inactive silica gel and chromatographed on a 3 × 40 cm column of silica gel (according to Pitra, 140 g, 0·10—0·25 mm). The silica gel was made by the Service Laboratories, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Praha - Lysolaje. Light petroleum with increasing content of ether (0—30%) was used as eluent. The composition of the fractions was monitored by thin-layer chromatography on silica gel as such, and on silica gel impregnated with silver nitrate[3]. The unresolved fractions were rechromatographed and the corresponding fractions combined with those from the main chromatographic run. Fraction 12 was dissolved in ether and esterified with an ether solution of diazomethane. The mixture was separated by chromatography on a column of silica gel in light petroleum–ether, with gradually increasing amount of ether (10 to 30%).

TABLE I

Survey of the Individual Instars in the Extracted Sample

Instar	Specimens		Fresh weight	
	number	%	mg	%
I	25	5·2	3·2	0·82
II	155	32·3	43·4	11·2
III apterous	145	30·2	95·7	24·6
III alate	11	2·3	6·8	1·75
IV apterous	79	16·5	120·1	30·9
IV alate	29	6·0	40·0	10·3
Imago apterous	28	5·8	67·8	17·4
Imago alate	8	1·7	11·8	3·03
Total	*480*	*100*	*388·8*	*100*

TABLE II

Total Composition of Lipid Fractions (%)

Hydrocarbons		C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}
Normal		+	+	+	+	+	0·1	+	0·3	0·2	0·9	2·2	19·8
Branched	A[a]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+[b]	+	0·1	0·4	2·0
	B[a]									+	+	0·8	
	C[a]									+	+		
	D[a]												

Esters	C_{32}	C_{34}	C_{36}	C_{38}	C_{40}	C_{42}	C_{44}	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}
	0·2	0·2	1·0	4·3	8·6	12·6	26·6	25·8	13·6	4·8	1·7	0·6

Hydrolysis Product of Esters	C_{12}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}
Alcohols		+	+	0·3	+	1·2	+	2·4	+	3·3	+	4·2
Acids[c]	+	1·4	+	14·1	+	7·2	+	13·7	+	2·8	+	1·7

Aldehydes	C_{22}	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}	C_{34}
	0·1	0·2	2·9	43·7	48·8	4·4	+

Saturated Glycerides	C_{29}	C_{31}	C_{33}	C_{35}	C_{37}	C_{39}	C_{41}
	0·1	0·2	0·4	6·5	84·2	7·8	0·8

Unsaturated Glycerides	C_{27}	C_{29}	C_{31}	C_{33}	C_{35}	C_{37}	C_{39}	C_{41}
	+	+	0·8	+	8·5	77·1	13·6	+

Free Fatty Acids[c]	C_{12}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{22}	C_{24}
Saturated	0·4	13·9		2·5	+	4·4		0·2	0·1	0·2
Monoenoic		0·3	+	6·1	+	23·4	2·5[d]			
Dienoic						46·0				

Free Alcohols	C_{20}	C_{22}	C_{24}	C_{26}	C_{27}	C_{28}	C_{30}	C_{32}
	+	0·3	1·0	18·4	0·3	69·1	10·3	0·6

Free α-Hydroxy Acids[c]	C_9	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}
Saturated	1·1	0·2	1·0	2·2	0·5	15·7		7·7		12·6	1·7	
Unsaturated					0·9		2·1	12·3	0·9	39·1		2·0

+ Traces; [a] designation of homologous series; [b] clasification into a homologous series not done;

TABLE II
(*Continued*)

C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{36}	C_{37}	C_{38}	C_{39}	Total
7·6	37·3	6·6	21·7	1·5	1·8	+	+	+				100
9·4	2·3	5·7	3·9	8·5	6·7	5·3	1·3	1·1	0·5	0·3		47·5
5·7		2·8	2·7	4·7	8·3	6·3	5·6	3·9	3·1	1·0	0·4	45·3
0·3			0·4		0·7	+	1·5	1·8	0·8	0·4		5·9
	0·2	0·4				0·3		0·4				1·3
												100

C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	
0·3	14·8	1·0	16·2	0·3	6·0	+	1·5	+	+	51·5
0·3	1·5	0·3	1·7	+	2·2	+	1·6	+	+	48·5
										100
										100
										100
										21·7
										32·3
										46·0
										100
										42·7
										57·3

[c] analyzed as methyl esters; [d] probably $C_{18:3}$ or $C_{18:4}$.

Treatment with molecular sieves[4]. 25 mg of paraffins (fraction 1) was refluxed in 1·5 ml 2,2,4-trimethylpentane[4] for 15 h in the presence of 0·75 g molecular sieve (Linde 5 Å). A total of 4·9 mg of a mixture of branched hydrocarbons was obtained.

Reesterification[5]. 4 mg of the fraction of esters or glycerides was heated in a sealed ampoule at 70°C for 3 h with 0·2 ml tetrachloromethane and 0·2 ml methanol, saturated with 5% gaseous HCl. After addition of bromothymol blue, the sample was neutralized with gaseous ammonia. After centrifugation of the precipitate, the solution was injected into a gas chromatograph either directly or after silylation.

Silyl derivatives. 2 mg of a sample (methyl esters of hydroxy acids and alcohols) was heated in a sealed ampoule with 0·1 ml acetonitrile and 0·1 ml bis(trimethylsilyl)trifluoroacetamide (Regisil) for 1 h at 150°C. The reaction mixture was injected directly into a gas chromatograph.

Acetates. 2 mg of a sample was heated in a sealed ampoule for 30 min to 50°C with 0·1 ml acetyl chloride. Excess acetyl chloride and hydrogen chloride were evaporated *in vacuo*.

Reduction of aldehydes with lithium aluminium hydride. 10 mg of fraction 6 was dissolved in 6 ml tetrahydrofuran and refluxed for 30 min with 40 mg $LiAlH_4$. After acidification with H_2SO_4 (1 : 20) and addition of 7 ml water, the reaction product was extracted with 3×5 ml ether. The combined extract was extracted with 3×3 ml water and dried with $MgSO_4$. A total of 9·7 mg primary alcohols was obtained.

Hydrogenation of glycerides. 9 mg of fraction 9 in 1·5 ml ethanol and 20 mg palladium catalyst (5% Pd on charcoal) was hydrogenated for 2 h at room temperature. After purification on a column ($0{\cdot}8 \times 6$ cm) of silica gel, a total of 7·8 mg product was obtained. This was analyzed by thin-layer chromatography, gas–liquid chromatography and mass spectrometry.

Hydroxylation of unsaturated acids[6]. 11 mg methyl esters of acids (fraction 12 C) in 2 ml of a mixture of ether and pyridine (8 : 1) was combined with 24 mg OsO_4 dissolved in 1 ml of the same mixture. After 2 h of standing, 60 ml of a freshly prepared suspension of Na_2SO_3 (prepared from 15 ml 16% aqueous solution of Na_2SO_3 and 50 ml methanol) was added. After 1 h of standing, the suspension was filtered. The solution was concentrated *in vacuo* under nitrogen atmosphere to about 10 ml and extracted with 3×7 ml chloroform[7]. The combined extracts were extracted with 2×7 ml water and dried with $MgSO_4$. A total of 7·8 mg methyl esters of saturated acids, dihydroxy acids and tetrahydroxy acids was obtained. The whole sample was silylated and analyzed by gas chromatography and mass spectrometry.

Gas chromatography was done on a Perkin-Elmer F 11 chromatograph and on a Pye Series 104 (Model 64) chromatograph, provided with flame-ionization detectors and a dual system of glass columns ($0{\cdot}3 \times 180$ cm and $0{\cdot}4 \times 150$ cm). For a qualitative evaluation, a linear relationship between the number of carbon atoms and the logarithm of the retention volume was used[8]. The quantitative evaluation of the chromatograms was done in such a way as to compare the products of the retention times and of the corresponding wave heights (without using correction factors). Hydrocarbons, aldehydes, glycerides, alcohols and their derivatives and methyl esters of hydroxy acids were chromatographed on 3% SE-30 placed on silanized Chromosorb G (100 to 120 mesh), the esters on 10% Dexsil 300 GC placed on Chromosorb W (100—120 mesh) and methyl esters of acids on 10% butanediol succinate on silanized Chromosorb W (100—120 mesh). n-Alkanes, alcohols and their derivatives and methyl esters of monocarboxylic acids were identified on the basis of identity of the retention data with those of synthetic standards. The number of carbon atoms in the individual peaks of esters and glycerides was determined on the basis of the corresponding retention data of synthetic standards. During gas chromatography of aldehydes the chromatograms contained at lower temperature of the injection block (at 200°C) some

unsymmetrical peaks which were not quantitatively reproducible. At higher temperatures of the injection block (270°C) this phenomenon was not observed and the quantitative results are in agreement with those obtained by gas chromatography of primary alcohols prepared by reduction of the aldehydes with $LiAlH_4$.

Mass spectrometry was done in a AEI MS 902 spectrometer connected with a Pye Series 104 (Model 64) chromatograph. The trimethylsilyl derivatives of methyl esters of hydroxy acids (prepared from fraction 12 F) were chromatographed on a 0·4 × 150 cm column containing 3% SE-30 on Gas-Chrom Q (100—120 mesh) and the mass spectra were obtained from the individual chromatographic peaks (electron energy 70 eV, ion source temperature 250°C). Glycerides (fractions 8 and 9) were injected directly into the mass spectrometer (electron energy 70 eV, ion source temperature 200°C).

Proton magnetic resonance was done on a Varian HA-100 spectrometer in deuteriochloroform using tetramethylsilane as standard. The chemical shifts are given in the δ-scale. PMR spectrum of unsaturated triglyceride (*II*): H_2 : 5·32 (quintett, 1 H, $J \simeq 5$ Hz). 2 H_1, 2 H_3: eight lines, AB — type, 4 H, $\nu_A = 4{\cdot}30$ (dd, H_1, H_3), $\nu_B = 4{\cdot}16$ (dd, H_1', H_3'); splittings: $J_{AB} = J_{1,1'} = J_{3,3'} \cong 12$ Hz, $J_{1,2} = J_{3,2} \cong 4{\cdot}5$ Hz, $J_{1',2} = J_{3',2} \cong 6$ Hz.

—O—CO—C$\underline{H}_2$—C$\underline{H}_2$—: 2·30 (t, 4 H, $J \cong 7$ H_2). H_α: 5·75 (bd, 1 H, $J_{\alpha,\beta} = 15{\cdot}2$, $J_{\alpha,CH_2} \neq 0$). H_β: 7·27 [m, 1 H, $\Sigma J = 25{\cdot}8$ Hz ($J_{\beta,\alpha} = 15$, $J_{\beta,\gamma} = 10$ from hexadeuteriobenzene solution)]. H_γ, H_δ: complex second-order multiplet in the region 5·95—6·35. C_δ — C$\underline{H}_3$: 1·85 (d, sec-order 3 H, $J \approx 4$ Hz). H_α, H_β: *trans*; H_β, H_γ: *trans*; H_γ, H_δ: not clearly established.

IR *spectroscopy* was done on a UR-10 (Zeiss, Jena) spectrophotometer in 0·01 cm cuvettes (6% solution in tetrachloromethane or in chloroform).

UV *spectroscopy* was done in a CF 4 (Optica Milano) spectrophotometer in a 1 cm cuvette (concentration in ethanol was $5{\cdot}03 \cdot 10^{-5}$ mol/l). In the range of 205—300 nm a λ_{min_1} was found at 218 nm (log ε 3·42) and a λ_{max_1} at 261 nm (log ε 4·34).

RESULTS

Using column chromatography on silica gel the whole extract A was separated into 12 fractions which were further analyzed by TLC, GLC and by IR, UV and mass spectra: Fraction 1 — hydrocarbons (4·2%), fractions 2, 3 and 4 — unidentified (traces), fraction 5 — esters (2·6%), fractions 6 — aldehydes (8·6%), fraction 7 — unidentified (0·2%), fraction 8 — saturated glycerides (14·2%), fraction 9 — unsaturated glycerides (12·9%), fractions 10 and 11 — unidentified (traces). Fraction 12 which contained predominantly a mixture of free acids and free alcohols and was poorly separated by adsorption chromatography, was separated only after esterification with diazomethane into five further fractions (A—F): fractions 12A and 12B — unidentified (0·4%), fraction 12C — free fatty acids (41·8%), fractions 12D — unidentified (3·6%), fraction 12E — free primary alcohols (9·4%), fraction 12F — free α-hydroxy acids (2·1%). A qualitative as well as quantitative review of all the identified compounds is in Table II.

Hydrocarbons: The first fractions eluted from the column with light petroleum contained only saturated hydrocarbons since their spectra displayed no absorption by double bonds and their thin-layer chromatography on silica gel impregnated with silver nitrate did not exhibit the presence of alkenes[3]. Gas chromatography showed that, in addition to n-alkanes, there is a relatively large amount of branched hydrocarbons. Molecular sieving separated the fraction of these branched hydrocarbons from the n-alkanes and the two fractions were analyzed separately by gas chromatography. The series of n-alkanes extends from C_{16} to C_{36} with the usual predominance of the odd-numbered homologues and a maximum of the C_{29} one. The chromatographic peaks of branched hydrocarbons are not symmetrical which suggests the complexity of the mixture

investigated. From a graphical plot[8] one can still derive two main homologous series A and B in the approximate range of $C_{24}-C_{39}$.

Esters: Fraction 5, absorbing in the IR region at 1174 and 1745 cm^{-1}, is formed by higher simple alkyl esters. The very weak absorption at 1690 cm^{-1} indicated that the esters might be accompanied by a minute amount of ketones. High-temperature[9] GLC proved the presence of a homologous series of even-numbered esters from C_{32} to C_{54} with maxima at C_{44} and C_{46}. For lack of material it could not be demonstrated unequivocally whether the individual chromatographic peaks correspond to individual compounds or to a mixture of isosteric esters[5]. After re-esterification with methanol the reaction mixture was again subjected to gas chromatography. The acidic components of the original esters form a homologous series of saturated normal monocarboxylic acids ($C_{12}-C_{34}$) and the neutral fraction is represented by a homologous series of saturated normal primary alcohols ($C_{14}-C_{34}$).

Aldehydes: In the IR spectrum, fraction 6 showed heavy absorption at 1728 cm^{-1} which corresponds to an aldehydic group. By reduction with lithium aluminium hydride, a mixture of primary alcohols was formed. Gas chromatography of both the original and the reduced fraction 6 indicates that it contains a homologous series of normal aldehydes from C_{22} to C_{34}.

Saturated glycerides: Fraction 8 absorbed in the IR spectrum at 1160 and 1743 cm^{-1} which corresponds to the presence of an ester bond. The R_F value during thin-layer chromatography corresponded to the R_F of synthetic triglyceride (trimyristin). Gas chromatography revealed the presence of a series of homologues, one of them heavily predominating. After re-esterification with methanol, gas chromatotraphy revealed glycerol plus methyl esters of saturated monocarboxylic acids, mainly C_6, C_{12}, C_{14} and C_{16}, with a predominance of C_{14}. The mass spectrum[10,11] of the whole fraction 8, which contained 84% triglyceride C_{37} and by employing the high resolution of the apparatus, molecular weight M^+ 610 (HR $C_{37}H_{70}O_6$) was found. The base peak was *m/e* 99 (HR $C_6H_{11}O{=}C_5H_{11}CO$); from this, CO is eliminated, giving rise to the metastable ion m* 50·9. Other characteristic fragments are *m/e* 592 ($M^+ - 18$, m* 574·5), *m/e* 495 ($C_{31}H_{59}O_4 = M^+ - OCOC_5H_{11}$), *m/e* 383 ($C_{23}H_{43}O_4 = M^+ - OCOC_{13}H_{27}$), *m/e* 285 ($C_{17}H_{33}O_3 = C_{13}H_{27}CO + 74$), *m/e* 211 ($C_{14}H_{27}O = C_{13}H_{27}CO$) and *m/e* 173 ($C_9H_{17}O_3 = C_5H_{11}CO + 74$). The mass spectrum shows further that the triglyceride is symmetrical since the fragment at *m/e* 481 [$M^+ - (OCOC_5H_{11} + 14)$] $\ll$ *m/e* 369 ($M^+ - CH_2OCOC_{13}H_{27}$) (Fig. 1*a*). On the basis of the results the main chromatographic peak C_{37} of this fraction has the structure of *I*. All the other chromatographic peaks of the homologous series contain triglycerides, differing always by two carbon atoms as demonstrated by comparing their retention times with those of the synthetic glycerides.

Unsaturated glycerides: Fraction 9 absorbed in the IR spectrum again at 1165 and 1743 cm^{-1}. The other wavenumbers at 1182, 1241, 1618, 1648 and 1722 cm^{-1} are typical of the presence of a doubly conjugated system of double bonds. Another absorption band at 1000 cm^{-1} suggests that the double bonds have a *trans* configuration. Gas chromatography revealed the presence of a homologous series of triglycerides, one of them again predominating. On the basis of the GLC products of re-esterification of fraction 9 with methanol, the other acid components of the triglycerides show a composition similar to that of the acids of fraction 8. Using the mass spectrum, a molecular mass of M^+ 606 was found. The base peak was *m/e* 99 (C_5H_7CO) from which CO is eliminated giving rise to a metastable ion m* 47·2. Other characteristic fragments are *m/e* 588 ($M^+ - 18$), *m/e* 495 ($M^+ - OCOC_5H_7$), *m/e* 379 ($M^+ - OCOC_{13}H_{27}$), *m/e* 285 ($C_{13}H_{27}CO + 74$) and *m/e* 211 ($C_{13}H_{27}CO$). The spectrum shows that the triglyceride contains two saturated acyls C_{14} and one acyl C_6 with two double bonds. Its position cannot be determined from the mass spectrum since both characteristic fragments $M^+ - (OCOC_5H_7 + 14)$ and $M^+ - (CH_2OCOC_{13}.H_{27})$ are minor components (Fig. 1*b*).

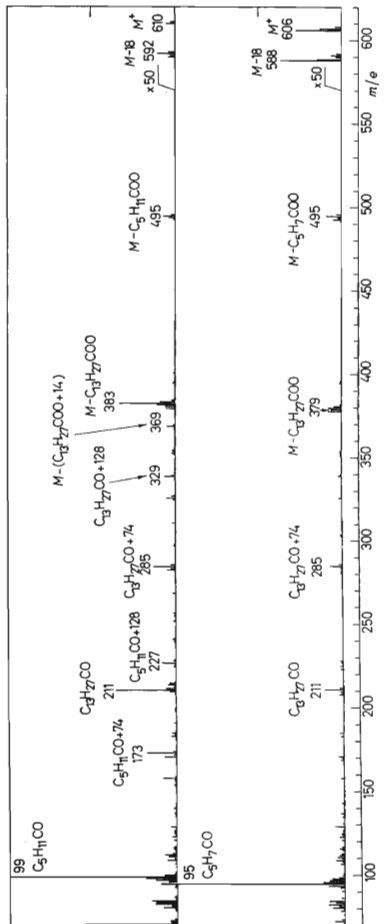
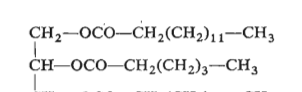


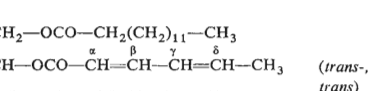
FIG. 1
Mass Spectrum of (*a*) Saturated Glycerides (fraction 8), (*b*) Unsaturated Glycerides (fraction 9)
For conditions see the experimental section.

From an analysis of a proton-magnetic resonance spectrum it was possible unequivocally to localize the conjugated system of double bonds into the shortest acyl residue C_6. Moreover, it was confirmed that both double bonds are *trans* and that the glyceride molecule is fully symmetrical.

Hydrogenation of fraction 9 yielded a mixture of saturated triglycerides which, according to the R_F values, retention data and mass spectrum, was identical with fraction 8. It thus follows from all the data obtained that the main triglyceride C_{37} has the structure of *II* and that the other chromatographic peaks of the homologous series again contain triglycerides differing always by 2 carbon atoms.



I



II (*trans*-, *trans*)

Free acids: The original chromatographic fraction 12 showed in its IR spectrum among others also absorption maxima at 940, 1287, 1414, 1711 and 2400–3400 cm^{-1} which are characteristic for the free carboxyl group. Analysis of the free acids was done in the form of their methyl esters which were obtained as a single chromatographic fraction 12 C after esterification of the original fraction 12 with diazomethane and after separation on a silica gel column. Thin-layer chromatography on silica gel impregnated with silver nitrate demonstrated that fraction 12 C contains saturated monoenoic and dienoic monocarboxylic acids. Their exact composition was shown by gas chromatography on a polar polyester phase. For the determination of the double bond positions the mixture was hydroxylated with osmium tetroxide and the hydroxy derivatives were silylated. From mass spectra prepared directly during gas chromatography, the positions of the double bonds could be determined unequivocally[7,12–14]. The homologous series of normal saturated, monocarboxylic acids extends from C_{12} to C_{24} with a maximum at C_{14}. The main representatives of acids with one double bond are 9-octadecenoic (*m/e* 215 and *m/e* 259) and 9-hexadecenoic (*m/e* 187 and *m/e* 259) acids. 9,12-Octadecadienoic acid represents in the mixture the doubly unsaturated acids (*m/e* 259, *m/e* 301 and *m/e* 387).

Free alcohols: Fraction 12 E absorbed in the IR spectrum at 1050 and 3615 cm^{-1} which are the frequencies typical of the hydroxyl group. The R_F values of this fraction were identical with the R_F values of the monoalcohols. It follows from the gas chromatography of the original, acetylated and trimethylsilylated fraction 12 E that we are dealing here with a homologous series of saturated unbranched even-numbered primary alcohols from C_{20} to C_{32} with a maximum at C_{28}.

Free hydroxy acids: In addition to absorptions typical of the $-COOCH_3$ group (1175, 1235 and 1740 cm^{-1}) the fraction 12 F showed in the IR spectrum absorption bands corresponding to the free hydroxy group (1082 and 3615 cm^{-1}). Gas chromatography of the silylated and nonsilylated fraction 12 F displayed the presence of two main homologous series A and B of methyl esters of hydroxy acids from C_9 to C_{21} with a maximum at C_{19} in series A. Mass spectra were recorded from the most highly represented peaks of silylated fraction 12 F. On the basis of characteristic fragments[12,15] $M^+ - 15$, $M^+ - 43$, $M^+ - 59$ and *m/e* 73, 75, 103 and 129 the studied peaks of homologous series B were identified as saturated α-hydroxy acids C_{13}, C_{15}, C_{17} and C_{19}, peaks from the A series as α-hydroxy acids C_{17} and C_{19} with one double bond (or monocyclic). Its position could not be determined for lack of material. In addition to the above characteristic fragments, all the mass spectra of these odd-numbered α-hydroxy acids contained a frag-

ment which arose from the mass $M^+ - 15$ by elimination of a neutral fragment of mass 118. This fragmentation was confirmed in all cases by a metastable ion. In addition, the spectra of saturated α-hydroxy acids contained a pronounced peak at *m/e* 176.

DISCUSSION

The main reasons that led us to the study of lipid compounds of aphids have been mentioned in the introduction. The chemical composition of the aphid lipids has been investigated only very unsystematically. The greatest number of references is to the presence of glycerides[16-22], or of fatty acids obtained by hydrolysis of these glycerides[23-27]. It is of interest that in most cases the principal acid component of the glycerides reported is myristic acid (C_{14}). As it was found now that the lipid extract contains substantial quantities (42%) of free acids[17,26] one must take into account separately the composition of bound acids (simple alkyl esters, glycerides, sterol esters) and of free acids. For this reason, one cannot use the composition of acids, obtained by saponification of the total lipid extract, for definitive conclusions on the composition of the glycerides.

Another important problem is whether the analyzed lipids were obtained only from the body surface or from whole crushed bodies. One can assume that the lipid compounds of aphids are concentrated mainly in the adipose body and in the wax cells which are modified hemocytes filled with wax-like substances and forming clusters near the siphunculus bases. Similarly, in the marginal tubercles of aphids compounds of this type are probably excreted and the function of these glandular organs can be somehow associated with the waterproofing integument of the aphids[28]. Recently, Eastop and Banks[1] obtained evidence that the function of the siphunculi and of the marginal tubercles is related to the resistance of *Myzus persicae* to insecticides. In *Acyrthosiphon pisum*, the marginal tubercles are only rudimentary or do not develop at all. On the other hand, the siphunculi are well developed. The analyzed extract A can thus contain compounds from the integument surface as well as from the wax cells, the content of which may exude from the siphunculi during manipulation with the aphids. It cannot be excluded that the fraction contains minor amounts of compounds extracted from the adipose body.

The lipids of *Acyrthosiphon pisum* have been mentioned so far only by Barlow[25]. The hydrolyzate of the lipid extract was analyzed by gas chromatography as to its content of fatty acids. In the context of our identifying the two main triglycerides it should be pointed out here that the unsaturated triglyceride *II* containing sorbic acid, was identified before in *Uroleucon* (= *Dactynotus*) *jaceae* by Bowie and Cameron[18] and in *Uroleucon* (= *Dactynotus*) *rudbeckiae* by Shimizu[22]. The last-named author also points out the potent fungicidal and yeast-inhibiting effect of sorbic acid and of its derivatives. A homologous triglyceride with sorbic acid, containing in positions 1,3 palmitic instead of myristic acid, was isolated from *Aphis nerii* by Brown, Cameron and Weiss[21]. It thus appears that the occurrence of these peculiar trigly-

cerides with sorbic acid has some function in the aphids. In addition to the above compound types, the literature mentions the presence of hydrocarbons, sterols and sterol esters[17], hydrocarbons and hydroxy acids(?)[26] and of hydrocarbons and alkyl esters[29].

The present communication is thus the first attempt at a systematic and detailed analysis of surface lipids of aphids where 8 groups of chemically distinct compounds were separated and identified in detail.

We are indebted to Mrs K. Matoušková and Mr P. Formánek for the recording of the IR and UV spectra and to Dr J. Smoliková for their interpretation, further to Dr Z. Samek for recording and interpreting the PMR spectrum. Our thanks are due to Mrs I. Dusová for careful technical assistance.

REFERENCES

1. Eastop V. F., Banks C. J.: Nature *225*, 970 (1970).
2. Sláma K.: Acta Soc. Ent. Czechoslov. *59*, 323 (1962).
3. Streibl M., Stránský K.: Fette, Seifen, Anstrichmittel *70*, 343 (1968).
4. O'Connor J. G., Burrow F. H., Norris M. S.: Anal. Chem. *34*, 82 (1962).
5. Streibl M., Jiroušová J., Stránský K.: Fette, Seifen, Anstrichmittel *73*, 301 (1971).
6. Wolff R. E., Wolff G., McCloskey J. A.: Tetrahedron *22*, 3093 (1966).
7. Perkins E. G., Argoudelis C. J.: Lipids *4*, 619 (1969).
8. James A. T., Martin A. J. P.: Biochem. J. *50*, 679 (1952).
9. Streibl M., Konečný K., Stránský K.: Fette, Seifen, Anstrichmittel *72*, 777 (1970).
10. Barber M., Merren T. O.: Tetrahedron Letters *1964*, 1063.
11. Sun K. K., Holman R. T.: J. Am. Oil Chem. Soc. *45*, 810 (1968).
12. Eglinton G., Hunneman D. H., McCormick A.: Org. Mass Spectr. *1*, 593 (1968).
13. Argoudelis C. J., Perkins E. G.: Lipids *3*, 379 (1968).
14. Capella P., Zorzut C. M.: Anal. Chem. *40*, 1458 (1968).
15. Capella P., Galli C., Fumagalli R.: Lipids *3*, 431 (1968).
16. Strong F. E.: Hilgardia *34*, 43 (1963).
17. Strong F. E.: Nature *202*, 622 (1964).
18. Bowie J. H., Cameron D. W.: J. Chem. Soc. *1965*, 5651.
19. Bowie J. H., Cameron D. E.: J. Chem. Soc. *C 1967*, 704.
20. Yurkiewicz W. J.: Entomol. News *80*, 85 (1969).
21. Brown K. S. jr, Cameron D. W., Weiss U.: Tetrahedron Letters *1969*, 471.
22. Shimizu Yu.: Naturwissenschaften *58*, 366 (1971).
23. Barlow J. S.: Nature *197*, 311 (1963).
24. Strong F. E.: Science *140*, 983 (1963).
25. Barlow J. S.: Can. J. Biochem. *42*, 1365 (1964).
26. Strong F. E.: Nature *205*, 1242 (1965).
27. Weiss U., Altland H. W.: Nature *207*, 1295 (1965).
28. Stroyan H. L. G.: J. Linn. Soc. London *43*, 644 (1958).
29. Dixon A. F. G., Martin-Smith M., Subramanian G.: J. Chem. Soc. *1965*, 1562.

Translated by A. Kotyk.

Note added in proof: On the basis of the recent observation that *trans*-β-farnesene is an aphid alarm pheromone [Science *177*, 1121 (1972)] we also identified this sesquiterpene additionally by GLC + MS in hydrocarbon fraction 2 (for details see the forthcoming paper).