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Colouring Matters of the Aphidoidea. XLIV*
A Survey of Long-Chain Acid Derivatives from Aphid Lipids Compared with Those of Related Insects. Glycerides of Octa-2,4,6-trienoic Acid

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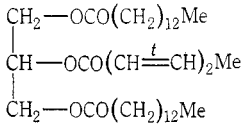
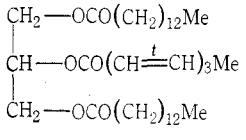
Abstract

A comprehensive survey of lipids representing all families of the Aphidoidea and several related families supports the chemotaxonomic separation of aphids and coccids from the remainder of the Hemiptera. Aphid glycerides are based chiefly on myristate, palmitate and sorbate as noted earlier; octa-2,4,6-trienoate residues are reported for the first time.

In Part XXV[1] Bowie and Cameron examined the fatty acid residues from a limited number of aphid triglycerides and noted two distinct differences from those of other insects. In agreement with an earlier survey[2] their dominant chain length corresponded to myristate or, occasionally, to palmitate, derivatives of eighteen-carbon acids being present to only a limited degree. In addition, they often contained sorbate, a constituent that has been found nowhere else in the animal kingdom. Thus 1,3-dimyristoyl-2-[(*E*,*E*)-sorboyl]glycerol (1) was by far the major lipid of the aphid *Dactynotus jaceae*. These unusual features do not appear to apply to the fatty acid residues in aphid phospholipids.[3]



(1)



(2)

Subsequent studies[4–7] have confirmed and extended these observations. Several families within the Aphidoidea have now been explored. Their lipid composition conformed to the pattern above and was differentiable from that of other divisions of the order Hemiptera. (The order Hemiptera is divided into two suborders Heteroptera and Homoptera, the Aphidoidea falling within the latter.)

* Part XLIII, *Aust. J. Chem.*, 1977, **30**, 2705.

[1] Bowie, J. H., and Cameron, D. W., *J. Chem. Soc.*, 1965, 5651.
[2] Strong, F. E., *Hilgardia*, 1963, **34**, 42.
[3] Cameron, D. W., and Drake, C. B., *Aust. J. Chem.*, 1976, **29**, 2723.
[4] Fast, P. G., *Prog. Chem. Fats Other Lipids*, 1970, **11**, 181.
[5] Thompson, S. N., *Comp. Biochem. Physiol. B*, 1973, **45**, 467.
[6] Greenway, A. R., Griffiths, D. C., Funk, C., and Prior, R. N. B., *J. Insect Physiol.*, 1974, **20**, 2423.
[7] Brown, K. S., *Chem. Soc. Rev.*, 1975, **4**, 263.

This paper develops these distinctions further. It is set against our interests in the quinonoid pigments and glycosides which are a characteristic feature of the Aphidoidea and Coccoidea but which are found in no other division of the Homoptera. From consideration of such compounds Banks and Cameron have concluded that aphids and coccids are chemically separable from the remainder of the Homoptera and particularly from the psyllids and aleyrodids, with which they are commonly associated.[8]

Total fats from several insect species were transesterified and their acid residues analysed by gas–liquid chromatography (g.l.c.) as methyl esters. The results are given in Table 1. Of the 41 species, 27 are members of the Aphidoidea. All nine families into which the Aphidoidea have commonly been subdivided[9] are included, three of them, the Greenideidae, Thelaxidae and Phylloxeridae, being examined for the first time. As seen from Table 1 all conform to the broad pattern outlined earlier and they differ strikingly from the other species listed.

The remaining species include representatives of all other major divisions of the Homoptera[10] except Fulgaromorpha and Peloridoidea. In particular the Psylloidea and Aleyrodoidea have been examined for the first time. Comparative data are also included for three species of bugs from within the suborder Heteroptera (superfamilies Notonectoidea, Corixoidea) and one species of thrip from the adjacent order Thysanoptera (superfamily Thripoidea).

With the exception of the Coccoidea these remaining groups conform broadly to one pattern, which differs from that of the Aphidoidea in being dominated by 18- and, to a lesser degree, 16-carbon acid residues. The same broad pattern is observed for most other orders of insects as well.[4,5] The clear conformity of psyllids and aleyrodids to this pattern is particularly interesting since it is consistent with other chemotaxonomic criteria discussed earlier but does not correlate with the common association of these insects with aphids and coccids.[8]

The Coccoidea resemble the Aphidoidea in containing unusually small proportions of 18-carbon acid residues. For the limited number of species examined the dominant chain length was 12, 14 or 16 carbons, together with appreciable proportions of 10. This preponderance of relatively short chain lengths was their most characteristic feature; there were no indications of conjugated unsaturated acids analogous to sorbic. Obviously more work on a wider range of coccid species would be useful, care being necessary to avoid complications arising from the copious waxy or resinous exudates which generally surround these insects. The conclusions reported here are, however, consistent with those of earlier workers.[4,11]

At family level this survey has covered the most comprehensive taxonomic range of aphids and related species so far examined. It clearly strengthens the chemotaxonomic basis for separation of aphids and coccids from the remainder of the Hemiptera.

In the course of this work we have noted the wide but non-uniform distribution of sorbate residues in fats from the Aphidoidea. In a few cases pure sorbate glycerides have actually been isolated, e.g. sorbodimyristin (1)[1] and the analogous sorbodipal-

[8] Banks, H. J., and Cameron, D. W., *Insect Biochem.*, 1973, **3**, 139.
[9] Kloet, G. S., and Hincks, W. D., 'A Check List of British Insects' Vol. 11, Part 1 (Royal Entomological Society: London 1964).
[10] Evans, J. W., *Annu. Rev. Entomol.*, 1963, **8**, 77.
[11] Hashimoto, A., Hirotani, A., Mukai, K., and Kitaoka, S., *Agric. Biol. Chem.*, 1970, **34**, 1839.

mitin[12] from *Dactynotus jaceae* and *Aphis nerii* (Aphididae) respectively; we have now isolated the former from *Tuberolachnus salignus* (Lachnidae) as well.

Table 1. Weight percentages of methyl esters from transesterification of insect lipids

Division	Family[A] (species)	Methyl esters (chain length:degree of unsaturation) (%)														
		4	6	8	6:2	10	12	8:3	14	14:1	16	16:1	18	18:1	18:2	18:3
Notonectoidea	1(*a*)	1	—	—	—	—	—	—	3	—	18	18	5	29	9	9
	1(*b*)	3	—	—	—	—	1	—	6	—	18	9	5	33	17	8
Corixoidea	2(*c*)	1	—	—	—	—	—	—	2	3	20	13	6	24	9	14
Cicadomorpha	3(*d*)	—	—	—	—	—	—	—	1	—	14	3	6	45	26	—
	4(*e*)	3	—	—	—	—	—	—	3	—	36	3	3	46	6	—
Psylloidea	5(*f*)	4	—	—	—	—	—	—	—	—	12	15	6	57	2	1
	5(*g*)	2	—	—	—	—	—	—	—	—	10	15	3	68	—	—
Aleyrodoidea	6(*h*)	2	—	—	—	—	—	—	1	—	21	1	1	68	3	1
	6(*i*)	—	—	—	—	—	—	—	2	—	21	15	5	46	—	—
Aphidoidea	7(*j*)	—	2	—	4	—	8	—	58	—	14	3	2	4	2	—
	7(*k*)	—	5	—	9	—	7	—	74	—	2	—	1	2	—	—
	8(*l*)	2	6	—	1	—	3	—	79	—	4	1	2	2	—	—
	8(*m*)	3	3	—	9	—	2	2	48	—	8	5	3	9	—	—
	8(*n*)	14	1	—	4	—	—	—	60	—	4	5	1	3	—	—
	8(*o*)	3	—	—	4	—	—	—	70	—	8	8	—	—	—	—
	9(*p*)	2	2	1	2	—	2	—	24	—	52	—	4	2	—	—
	9(*q*)	12	—	—	1	—	2	—	47	—	13	3	3	9	3	—
	10(*r*)	—	2	—	11	—	1	—	52	—	30	—	2	1	1	—
	11(*s*)	3	3	—	—	—	1	—	31	—	54	—	2	1	—	—
	11(*t*)	1	4	—	—	—	1	—	52	—	36	—	2	2	1	—
	11(*u*)	—	—	—	1	—	5	1	63	4	6	4	2	6	1	—
	11(*v*)	7	10	1	1	—	7	—	55	—	7	3	1	1	1	—
	11(*w*)	—	3	—	—	—	2	—	65	5	6	4	2	7	2	—
	11(*x*)	1	3	—	2	—	3	1	63	2	6	2	4	7	6	—
	11(*y*)	8	6	—	1	—	1	—	33	—	41	—	2	2	—	—
	11(*z*)	1	1	—	1	—	5	4	55	—	5	5	6	12	—	—
	11(*aa*)	—	—	—	1	—	1	1	16	—	72	—	4	3	—	—
	11(*bb*)	8	8	—	1	—	4	—	70	—	4	—	2	—	—	—
	12(*cc*)	5	6	—	2	—	9	—	58	—	14	—	3	—	—	—
	12(*dd*)	4	3	—	—	—	11	[B]	48	—	22	—	5	5	—	—
	12(*ee*)	6	10	—	—	—	6	1	39	1	22	—	6	3	2	—
	13(*ff*)	1	—	8	—	—	4	[B]	46	—	24	1	9	4	3	—
	13(*gg*)	1	—	7	—	—	9	[B]	63	2	9	1	2	4	—	—
	14(*hh*)	—	3	—	—	—	9	—	65	—	17	2	4	—	—	—
	15(*ii*)	11	4	1	—	—	—	—	13	—	43	8	8	4	2	—
	15(*jj*)	1	1	—	—	—	5	—	64	—	20	—	—	—	—	—
Coccoidea	16(*kk*)	4	—	—	—	5	24	—	24	—	41	—	1	1	—	—
	17(*ll*)	1	—	1	—	17	46	—	24	—	—	—	6	1	4	—
	18(*mm*)	10	—	—	—	4	34	—	17	—	3	3	29	—	—	—
	18(*nn*)	1	—	—	—	21	4	—	63	—	3	—	4	3	—	—
Thripoidea	19(*oo*)	—	—	—	—	—	—	—	1	—	27	3	4	44	16	5

[A] Key to individual families and species is given in the Experimental section under Analytical Results.
[B] Component detected in abundance of less than 0·5% (see Discussion).

Sorbate residues were most easily detected by strong absorption maxima in the region of 260 nm even for species whose lipid fractions were contaminated by aphid pigments or carotenoids,[8] in which case further fractionation by thin-layer chromatography was required. Several species were thus observed to contain a new component absorbing near 300 nm. Generally this accompanied larger amounts of sorbate but in three species *Pemphigus bursarius*, *P. spirothecae* (Pemphigidae) and *Phloeomyzus redelei* (Thelaxidae) no sorbate was detected. For purposes of purification its absence was an advantage and accordingly the two *Pemphigus* species were chosen for further fractionation, even though higher proportions of the new component were present in some of the sorbate-containing species.

[12] Brown, K. S., Cameron, D. W., and Weiss, U., *Tetrahedron Lett.*, 1969, 471.

The new component was formulated as a glyceride of (*E*,*E*,*E*)-octa-2,4,6-trienoic acid, i.e. a vinylogue of sorbic acid. G.l.c. analysis of relevant aphid fats after transesterification showed a constituent indistinguishable from synthetic methyl octatrienoate. Comparison at the triglyceride level was more difficult, however. Careful chromatography of crude fat from *P. bursarius* achieved separation of a strongly absorbing glyceride representing *c.* 1% of the total. In light absorption and t.l.c. behaviour it was indistinguishable from the dimyristoyloctatrienoylglycerol (2), which was synthesized analogously to (1).[1] However, the natural glyceride could not be obtained homogeneous. Transesterification and g.l.c. analysis showed that, although the major components were myristate and octatrienoate, there were also significant contaminating proportions of palmitate and laurate. It appears that, although the chromatographic system could separate unsaturated from saturated triglycerides, further fractionation of the former into components of different chain lengths was impractical unless one such component predominated to the point where fractional crystallization became effective. Direct hydrolysis of a similar glyceride fraction from *P. spirothecae* gave octatrienoic acid itself.

Glycerides of octatrienoic acid, unlike those of sorbic acid, were never observed to represent more than a minor proportion of total aphid fat, their high extinction values permitting detection even in minute amounts. However, it is worth noting that they are also unstable. The synthetic compound (2), for example, decomposed extensively on standing, the process presumably involving the conjugated acyclic system. This might inhibit accumulation of natural material in the insect in substantial quantity.

Octatrienoic acid has not previously been observed as a glyceride constituent. Along with several other unsaturated systems it has been alluded to in the mass spectrometric fragmentation of glycerides of *Acyrthosiphon pisum* (Aphididae)[13] but no isolation appears to have been carried out. Methyl octatrienoate has been reported from microbial sources.[14,15]

Given the intriguing situation in which the occurrence of sorbate and octatrienoate residues appears to be localized in glycerides of the Aphidoidea we have explored the possible occurrence of higher and lower vinylogues as well. Neither mono- nor tetraenoate chromophores could be detected spectroscopically from a very wide range of species.[16] Moreover, the lower vinylogue, crotonate, was not observed in the present work, even though the g.l.c. system could have detected it. The distribution of sorbate and octatrienoate within the Aphidoidea is clearly complex and, though areas of correlation exist, a complete picture would require very searching analysis indeed.

Experimental

Most of the species were collected in the vicinity of Cambridge, U.K., and much of the work described herein was carried out in the Cambridge University Chemical Laboratory.

Extraction and Analysis of Lipids

Insects (0·01–1 g depending on availability) were extracted and the separated ether fraction obtained according to the literature.[8] The yield of crude lipid after evaporation of solvent was generally 3–15%.

[13] Stransky, K., Trka, A., Kohoutova, J., and Streibl, M., *Collect. Czech. Chem. Commun.*, 1976, **41**, 1977.
[14] Kamal, A., Haider, Y., Akhtar, R., and Qureshi, A. A., *Pak. J. Sci. Ind. Res.*, 1971, **14**, 63.
[15] Kamal, A., Akhtar, R., and Qureshi, A. A., *Pak. J. Sci. Ind. Res.*, 1971, **14**, 71.
[16] Banks, H. J., Ph.D. Thesis, Cambridge, 1969.

In a typical transesterification crude lipid (6 mg) was dissolved in boiling dry methanol (5 cm^3). A 0·1 M solution of sodium methoxide in dry methanol (1 cm^3) was added and boiling maintained for 2 min, after which water (15 cm^3) was added and the pH brought to 6·8 with dilute hydrochloric acid. The resulting milky suspension was extracted with ether (3 × 5 cm^3); the combined extracts were washed with water and dried, and then concentrated to 0·2 cm^3 by gentle heating in a microdistillation apparatus. The concentrate was then chromatographed on a 15% L.A.C. column (2 m) at 80° (C_4–C_{10}) and 160° (C_8–C_{18}). Quantities were estimated by comparison with standard mixtures of synthetic methyl esters. Standard mixtures were also used to establish that there was no loss of the lower-boiling esters during concentration of extracts by distillation.

Analytical Results

These are given in Table 1. Limited proportions of additional components of unassigned structure were observed for a number of species. These components have been omitted from the Table but have been estimated in the calculation of weight percentages. *Molar* percentages for the lower-molecular-weight residues would be appreciably higher than those given in the Table; for the higher-molecular-weight residues there would be a slight lowering. Proportions of less than 0·5% have not been noted except for octatrienoate. Esters are listed in order of increasing retention time.

The nomenclature and order of precedence used in Table 1 correspond to those of Kloet and Hincks[9] for species found in Britain. Australian species have been interpolated.

The key to individual families in the Table is as follows: 1 Notonectidae; 2 Corixidae; 3 Cercopidae; 4 Cicadellidae; 5 Psyllidae; 6 Aleyrodidae; 7 Lachnidae; 8 Chaitophoridae; 9 Callaphididae; 10 Greenideidae; 11 Aphididae; 12 Thelaxidae; 13 Pemphigidae; 14 Adelgidae; 15 Phylloxeridae; 16 Coccidae; 17 Eriococcidae; 18 Pseudococcidae; 19 Thripidae.

The key to individual species in the Table is as follows: (*a*) *Notonecta obliqua* Gallen; (*b*) *Notonecta maculate* Fabricius; (*c*) *Corixa* sp. Geoffroy; (*d*) *Philaenus spumarius* (L.); (*e*) *Eupteryx melissae* Curtis; (*f*) *Psylla buxi* (L.); (*g*) *Psylla mali* Schmidberger; (*h*) *Aleyrodes proletella* (L.); (*i*) *Trialeurodes vaporariorum* (Westwood); (*j*) *Cinara abieticola* (Cholodkovsky); (*k*) *Tuberolachnus salignus* (Gmelin); (*l*) *Periphyllus testudinaceus* (Fernie); (*m*) *Chaitophorus beuthani* (Börner); (*n*) *Chaitophorus capreae* (Mosley); (*o*) *Chaitophorus tremulae* Koch; (*p*) *Callaphis juglandis* (Goeze); (*q*) *Myzocallis coryli* (Goeze); (*r*) *Greenidea ficicola* (Takahashi); (*s*) *Hyalopterus pruni* (Geoffroy); (*t*) *Aphis fabae* Scopoli; (*u*) *Elatobium abietinum* (Walker); (*v*) *Cavariella aegopodii* (Scopoli); (*w*) *Myzus cerasi* (Fabricius); (*x*) *Myzus persicae* (Sulzer); (*y*) *Rhopalosiphoninus calthae* (Koch); (*z*) *Acyrthosiphon pisum* (Harris); (*aa*) *Megoura viciae* Buckton; (*bb*) *Wahlgreniella arbuti* (Davidson); (*cc*) *Anoecia corni* (Fabricius); (*dd*) *Mindarus abietinus* Koch; (*ee*) *Phloeomyzus redelei* Hille Ris Lambers; (*ff*) *Pemphigus bursarius* (L.); (*gg*) *Pemphigus spirothecae* Passerini; (*hh*) *Adelges* (*Sacchiphantes*) *abietis* (L.); (*ii*) *Phylloxerina salicis* (Lichtenstein); (*jj*) *Phylloxera glabra* (von Heyden); (*kk*) *Saissetia coffeae* (Walker); (*ll*) *Eriococcus coriaceus* (Maskell); (*mm*) *Planococcus citri* (Risso); (*nn*) *Pseudococcus fragilis* Brain; (*oo*) *Taeniothrips inconsequens* (Uzel).

1,3-Dimyristoyl-2-[(E,E)-*sorboyl*]*glycerol*

Crude fat (500 mg) from *Tuberolachnus salignus* was chromatographed on silicic acid as in the literature[1] and the u.v.-absorbing fractions worked up to yield *1,3-dimyristoyl-2-*[(E,E)-*sorboyl*]*glycerol* (102 mg), m.p. and m.m.p. 54–54·5°, identical with authentic material[1] in u.v., i.r. and p.m.r. spectra and in chromatographic behaviour. Transesterification afforded methyl myristate and methyl sorbate as the only products.

1,3-Dimyristoyl-2-[(E,E,E)-*octa-2,4,6-trienoyl*]*glycerol*

A suspension of octa-2,4,6-trienoic acid (3 g) in light petroleum (50 cm^3) was treated dropwise with thionyl chloride (10 g) and the mixture boiled for 3 h. Solvents were removed under vacuum. The residue was heated with a solution of 1,3-dimyristoylglycerol[1] (1 g) in pyridine (5 cm^3) for 3 h at 60°. The solution was then treated carefully with cold dilute sulfuric acid (100 cm^3), the mixture extracted with ether and washed well with aqueous sodium bicarbonate to give *1,3-dimyristoyl-2-*[(E,E,E)-*octa-2,4,6-trienoyl*]*glycerol* (200 mg) (2). It was purified by t.l.c. on silica (hexane/ether, 4 : 1) and by recrystallization from methanol, m.p. 61–62° (Found: C, 73·8; H, 10·8. $C_{30}H_{68}O_6$ requires C, 73·9; H, 10·8%). λ_{max} (EtOH) (log ε) 307 (4·54) nm. ν_{max} (KBr) 1730, 1718, 1642, 1618

cm^{-1}. δ (CCl_4) $SiMe_4$ as internal reference 0·88 (6H, m, CH_3CH_2); 1·26 (44H, m, CH_2); 1·84 (3H, d, J 6 Hz, CH_3CH); 2·30 (4H, m, CH_2CO); 4·10 (4H, m, CH_2O); 5·18 (1H, m, CHO); 5·80–6·50 (m, 5H, vinylic CH); 7·22 (1H, m, CHCO).

The product fluoresced purple in u.v. light, R_F 0·73 on silica gel GF_{254} (hexane/ether, 2 : 1). Transesterification and analysis by g.l.c. afforded methyl myristate and methyl octatrienoate as the only products.

Octatrienoyl Glycerides from Pemphigus bursarius

The insects (30 g) were collected by opening the appropriate galls from poplars. They were ground successively with acetone (thrice), ether (twice), methanol containing acetic acid (1%) and ethanol. The residue was then suspended in water (250 cm^3) containing acetic acid (5 drops) and extracted with butanol. The combined organic extracts were evaporated under vacuum. The residue was then partitioned between ether and water pH 3. The former layer gave yellowish brown crude fat (3 g). This was chromatographed in two portions on a column of silicic acid (40 by 5 cm) in hexane/ether (20 : 1 to 10 : 1). Fractions (25 cm^3) were collected. Fractions 19–25 were yellow, containing carotenoids. Fractions 30–65, monitored by electronic absorption, were evaporated. The residue was recrystallized three times from methanol, subjected to preparative t.l.c. and one further recrystallization to give octatrienoyl glycerides (2·5 mg), m.p. 59–60°. λ_{max} ($\log\varepsilon$) 305 (4·53) nm. The product was indistinguishable from (2) on t.l.c. but on transesterification gave indications of significant proportions of C_{12} and C_{16} residues.

Octatrienoic Acid from P. spirothecae

P. spirothecae (65 g) were collected by opening the appropriate galls from poplars. They were extracted and fractionated as above to give crude octatrienoyl glycerides. Without crystallization these were boiled for 3·5 h with aqueous methanolic sodium hydroxide (2·5 M). After evaporation of methanol and acidification the mixture was extracted with ether. The extracts were re-extracted with aqueous sodium bicarbonate. The recovered acids were fractionally crystallized to give octa-2,4,6-trienoic acid from ethanol, m.p. 175°. It was undepressed in admixture with authentic material, m.p. 186°, but insufficient was available to permit crystallization to constant melting point. However, the two samples had identical u.v. and i.r. absorption and chromatographic behaviour.

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