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Colouring Matters of the Aphidoidea. XLI* The Phospholipids of *Eriosoma lanigerum* (Hemiptera : Insecta)

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Abstract

Whereas triglycerides of the woolly aphid *E. lanigerum* are rich in myristate and sorbate residues, the phospholipids contain chiefly oleate, linoleate and stearate. In agreement with other aphid work, there is significantly more phosphatidyl ethanolamine than phosphatidyl choline.

Triglycerides and phospholipids of the Aphidoidea differ remarkably from those of other insects, including other members of the Hemiptera. The dominant fatty acid residue of the triglycerides is myristate (C_{14}).[1–7] Some species also contain significant proportions of sorbate.[2,3,6,7] The latter has not been found anywhere else in the animal kingdom.

Only a small number of aphid phospholipids have been examined[4,8] but the dominant chain length of the fatty acids was C_{18} as for most other insects. Unusually however, there was a significantly lower proportion of phosphatidyl choline (lecithin) than phosphatidyl ethanolamine.

Fast[4] has noted that no aphid species containing substantial proportions of sorbate triglycerides has yet been examined for phospholipids. The status of analogous sorbate-containing phospholipids is thus undetermined. This Communication is concerned with such an examination.

Triglycerides of the woolly apple aphid *Eriosoma lanigerum* Hausmann (Pemphigidae) contain well over half their weight of myristate.[1,2,4,5] Bowie and Cameron[2] reported that there was also a small proportion (2%) of sorbate. However, reexamination of the original sample has shown this to be a considerable underestimate. The $E_{1\,cm}^{1\%}$ value at the sorbate absorption maximum (261 nm) was in error by a factor of 10 and should be revised to 407. Since the $E_{1\,cm}^{1\%}$ value of pure sorbodimyristin is 402 this represents about one sorbate unit per mole of triglyceride and a corrected proportion of 18% of sorbate by weight.

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E. lanigerum is thus extraordinarily rich in sorbate triglycerides. This is supported by t.l.c. examination of the crude fat and by ultraviolet[9] and mass spectrometric[6] analysis of independently collected samples. *E. lanigerum* should therefore be a suitable species for assessing the presence of sorbate-containing phospholipids.

The phospholipids, which amounted to 1% of total lipids, were separated from triglycerides by solvent partitioning and by chromatography on silicic acid. Ultraviolet spectrophotometry showed that, weight for weight, they could contain at most 4% of the proportion of sorbate present in the triglycerides. This upper limit would require the unlikely assumption that all the measured absorption at 261 nm was due to sorbate residues and that there was no contamination from the strongly ultraviolet-absorbing triglyceride fraction. No sorbate-containing phospholipids were evident on chromatographic examination.

Transesterification of total phospholipids and analysis of the resulting methyl esters by coupled g.l.c.–m.s. showed the presence of myristate (10%), palmitate (6%), palmitoleate (8%), stearate (13%), oleate (41%) and linoleate (21%) as the major components. No sorbate was detected. This observation together with the high proportions of C_{18} acid residues and the limited amounts of C_{14} afford a marked contrast with hydrolysates from the triglyceride fraction.[1,2,4,5]

Microquantities of the phospholipids were separated into five phosphorus-containing fractions by careful t.l.c. in two different solvent systems. The first, second and fourth, in order of decreasing R_F values, were identified alongside authentic samples of phosphatidyl ethanolamine, phosphatidyl choline and lysophosphatidyl choline respectively. The third and fifth were inferred to be lysophosphatidyl ethanolamine and phosphatidyl serine (or possibly lysophosphatidyl serine) from R_F values.[10] Only the first, third and fifth were Ninhydrin-positive, consistent with these assignments.

Estimation of each of the five components gave weights of phosphorus in the ratio 190 : 70 : 140 : 75 : 5 respectively. There was thus *c.* 2·7 times as much phosphatidyl ethanolamine as phosphatidyl choline. This is in qualitative agreement with Fast's observations on other aphid phospholipids.[4,8]

E. lanigerum has thus afforded a striking example of differences in the acyl residues of triglycerides and phospholipids within the same species. These differences underline the differing biochemical roles[4] of the two classes of lipids.

Experimental

All insect extractions were carried out at 4°. Coupled g.l.c.–m.s. data were obtained on a Perkin–Elmer 270B instrument.

Preliminary Separation of Phospholipids of E. lanigerum

Freshly collected insects (67 g)[11] were ground in a mortar with sand (10 g) and phosphate buffer (70 cm³, pH 6·6) and allowed to stand at 0° for 1 h. The buffer was then removed after centrifugation and the process was repeated. (The buffer extracts were retained for enzyme studies.[12])

The insect bodies were then macerated with acetone (250 cm³), filtered and re-extracted with acetone (250 cm³) and ether (250 cm³). Finally they were suspended in methanol containing acetic acid (1%) (150 cm³), this extract being allowed to stand for several hours at 0°. The combined

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extracts were filtered and solvents removed by freeze-drying. The residue was then partitioned between ether (150 cm^3) and water (150 cm^3), centrifugation being required to separate the phases. The aqueous and interfacial phases were re-extracted with ether (3×25 cm^3), the combined ether extracts washed with water (25 cm^3) and freeze-dried to give a residue (6·3 g). This was partitioned between water–methanol (2 : 15) (170 cm^3) and petroleum (150 cm^3) to give a preliminary separation of phospholipids (methanol phase) from triglycerides (petroleum phase). The former phase was freeze-dried and the crude extract (120 mg) dissolved in chloroform (10 cm^3) and stored at $-60°$. The solution was bright red in colour owing to the presence of small quantities of aphin pigments.[7,12]

Analysis of Phospholipids

(A) The foregoing chloroform solution (3·3 cm^3) was chromatographed on silicic acid (15 g). Successive elution with chloroform (150 cm^3) and with acetone (50 cm^3) gave neutral lipid (16 mg) and the aphin pigments respectively. The column was then eluted with aliquots (6×20 cm^3) of acetone–methanol (19 : 1; 17 : 3; 14 : 6; 12 : 8; 6 : 14; 3 : 17 respectively). The phospholipids (22 mg) were eluted by the last two aliquots. After evaporation of solvent they were redissolved in ethanol and their u.v. absorption at 261 nm compared with that of neutral lipid.

(B) The foregoing chloroform solution (2 cm^3) was chromatographed in chloroform on silica gel GF_{254} plates (0·05 cm), prepared in sodium carbonate solution (1 mM). Neutral lipids ran at the solvent front, phospholipids and aphin pigments remaining at the origin. After removal of the neutral lipids the plates were rerun in chloroform–methanol–water (14 : 6 : 1). The five molybdate-positive bands were viewed under u.v. light and each eluted successively with chloroform–methanol–acetic acid–water (50 : 25 : 8 : 4; 20 cm^3) methanol (20 cm^3) and methanol–acetic acid–water (94 : 1 : 5; 20 cm^3). The combined extracts were evaporated at 25° and compared with authentic phospholipids in two chromatographic systems: (i) SiO_2 (H) (0·05 cm), Na_2CO_3 (1 mM) in chloroform–methanol–water (14 : 6 : 1 v/v). (ii) SiO_2 (GF_{254}) (0·05 cm), Na_2CO_3 (1 mM) in chloroform–methanol–acetic acid–water (50 : 25 : 8 : 4 v/v). Each was also subjected to total phosphorus analysis and structures assigned as in the Discussion. R_F (system (i)), R_F (system (ii)) and phosphorus content (μg) were as follows: 0·57, 0·75, 190; 0·49, 0·67, 70; 0·44, 0·62, 140; 0·38, 0·56, 75; 0·25, 0·49, 5.

(C) The foregoing chloroform solution (1 cm^3) was subjected to t.l.c. as in (B) and the combined phospholipids redissolved in dry methanol (7 cm^3). This solution was boiled with methanolic sodium methoxide (0·1 M, 1 cm^3) for 2 min. Water (15 cm^3) was then added and the pH adjusted to 6·8 with hydrochloric acid (0·1 M). The mixture was extracted with ether (3×15 cm^3), the combined extracts washed with water, dried and solvent evaporated below 20°. The resulting methyl esters were subjected to g.l.c.–m.s. (stainless steel 6 ft by $\frac{1}{4}$ in., 5% SE30 on Chromosorb W (60/80), 100–265°, 5°/min, helium, flow rate 45 instrumental units). The components, described in the Discussion, were identified by retention time against authentic materials and by mass spectrometric fragmentation.

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