Artifacts produced by acidic hydrolysis of lipids containing 3-hydroxyalkanoic acids

Stephen G. Wilkinson

Department of Chemistry, The University, Hull HU6 7RX, England

Summary After acidic hydrolysis of lipid A preparations from pseudomonads, products containing both hydroxy and nonhydroxy fatty acids were obtained. The major products were alkanoate esters of the hydroxy acids. Similar compounds were formed when a 3-hydroxy acid was heated with a nonhydroxy acid under the same conditions.

Supplementary key words bacterial lipopolysaccharides · lipid A · pseudomonads · linked fatty acids

Fatty acids can be released for analysis by either acidic or alkaline hydrolysis of lipids. Although similar results may be obtained by either method, acidic hydrolysis is often preferred for lipids that contain amide-bound fatty acids. Prominent among such lipids are the lipid A fractions of bacterial lipopolysaccharides, which characteristically contain both alkanoic and 3-hydroxyalkanoic acids.

The determination of fatty acid compositions of lipid A preparations is complicated by the partial dehydration of 3-hydroxy acids under acidic conditions. Other complications were suspected when erratic results, particularly for hydroxy acids, were obtained for acidic hydrolysates of lipid A preparations from *Pseudomonas aeruginosa* and *P. alcaligenes* (1). To investigate this problem, the ether-soluble products from hydrolysis of the lipids with 4 N HCl at 105°C for 5 hr were treated with diazomethane and examined by TLC (*d* and *e* in Fig. 1). In addition to spots corresponding to the methyl esters of hydroxy acids, alkanoic acids, and 2-alkenoic acids, each hydrolysate gave at least three other spots (*P1*, *P2*, and *P3* in Fig. 1), of which the intensities decreased with R_F values. These patterns closely resembled those found for a similar hydrolysate of an ornithine-containing lipid from *P. rubescens*, which also contains 3-hydroxy and nonhydroxy acids (2). The unexpected products from the latter lipid contained both classes of fatty acids, and this result was also obtained for product P1 from the lipid A of *P. aeruginosa* (isolated by preparative TLC and subjected to acidic methanolysis).

To check on the formation of artifacts, mixtures of hexadecanoic acid with either 3-hydroxydodecanoic acid or 3-hydroxyhexadecanoic acid (about 2 mg of each component) were treated with 4 N HCl (2 ml) at 105°C for 5 hr. After treatment with diazomethane, the ether-soluble

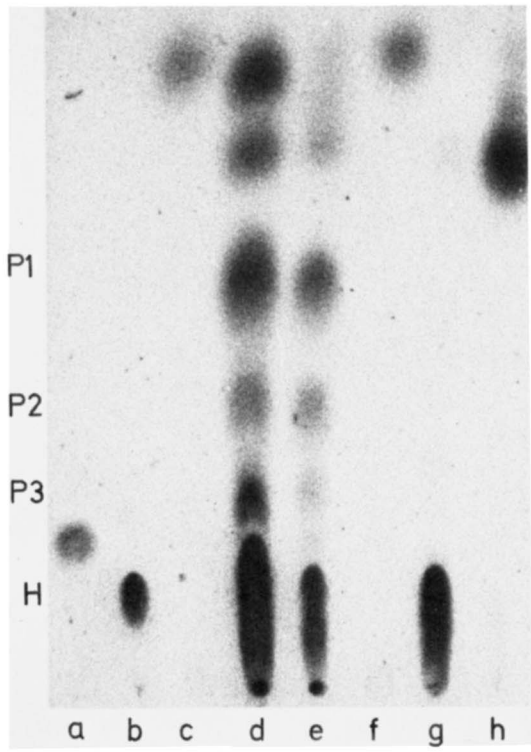

Fig. 1. Thin-layer chromatogram of esterified products from acidic hydrolysates of lipid A preparations from *P. aeruginosa* and *P. alcaligenes*. Separations were carried out using silica gel G (Merck) and dichloromethane as solvent; spots were detected by charring (6). Methyl esters from: *a*, 2-hydroxydodecanoic acid; *b*, 3-hydroxydodecanoic acid; *c* and *f*, hexadecanoic acid; *d*, acids in *P. aeruginosa* hydrolysate; *e*, acids in *P. alcaligenes* hydrolysate; *g*, HCl-treated 3-hydroxydodecanoic acid; *h*, 2-dodecenoic acid (major spot). *P1*, *P2*, *P3*, artifacts of hydrolysis; *H*, hydroxy ester region.

products gave TLC results comparable with those obtained for lipid A hydrolysates. The products with the same mobility as P1 were the hexadecanoate esters of the methyl 3-hydroxyalkanoates. These compounds had the infrared spectra of fatty esters and were decomposed by acidic methanolysis (3) to the methyl esters of the component acids. The compound derived from hexadecanoic acid and 3-hydroxyhexadecanoic acid gave the same mass spectrum as a synthetic sample of the hexadecanoate ester of methyl 3-hydroxyhexadecanoate (2). The spectrum contained a molecular ion peak (*m/e* 524) and a small peak at *m/e* 493 (M−31) due to loss of the methoxy group. The most abundant high-mass ions, *m/e* 268 and 269, corresponding to loss of hexadecanoic acid and hexadecanoate, respectively, apparently gave rise to ions at *m/e* 236 and 237 through loss of methanol. Other significant peaks occurred at *m/e* 341 (analogous to the *m/e* 103 peak for methyl esters of 3-hydroxy acids [4]), 328 (possibly the product from a McLafferty rearrangement), 285 (loss of the hexadecanoyl group), and 239 (the acylium ion from the hexadecanoate residue). Analogous fragment ions were revealed by the mass spectrum of the compound de-

Abbreviations: TLC, thin-layer chromatography.

rived from hexadecanoic acid and 3-hydroxydodecanoic acid.

No products resembling P1–3 were found when 3-hydroxydodecanoic acid alone was treated similarly with HCl and then esterified (*g* in Fig. 1). However, the streak in the hydroxy ester region on TLC (*H* in Fig. 1) suggested that polyesters (5) could have been formed. By using methyl hexadecanoate as an internal standard, it was confirmed that the amount of methyl 3-hydroxydodecanoate was about doubled by acidic methanolysis of the total products. Thus, components such as P2 and P3 in lipid hydrolysates could correspond to dimers and higher oligomers of 3-hydroxyalkanoic acids that were further esterified with other fatty acids. In the model experiment with hexadecanoic acid and 3-hydroxydodecanoic acid, the amounts of these acids incorporated into artifact products were 21% and 50%, respectively (determined by using methyl pentadecanoate as an external standard).

These experiments demonstrate that acid-catalyzed polymerization of a 3-hydroxy acid and esterification between hydroxy and nonhydroxy acids can occur (presumably in the lipid phase) under nominally hydrolytic conditions. It is clear that structural conclusions (2) based on the results of such experiments are invalid and that fatty acid contents and compositions determined after acidic hydrolysis of lipids containing 3-hydroxyalkanoic acids may be seriously in error.

Manuscript received 2 July 1973; accepted 13 November 1973.

REFERENCES

1. Drewry, D. T., J. A. Lomax, G. W. Gray, and S. G. Wilkinson. 1973. Studies of lipid A fractions from the lipopolysaccharides of *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes*. *Biochem. J.* **133:** 563–572.
2. Wilkinson, S. G. 1972. Composition and structure of the ornithine-containing lipid from *Pseudomonas rubescens*. *Biochim. Biophys. Acta.* **270:** 1–17.
3. Stoffel, W., F. Chu, and E. H. Ahrens, Jr. 1959. Analysis of long-chain fatty acids by gas–liquid chromatography. Micromethod for preparation of methyl esters. *Anal. Chem.* **31:** 307–308.
4. Ryhage, R., and E. Stenhagen. 1960. Mass spectrometric studies. VI. Methyl esters of normal chain oxo-, hydroxy-, methoxy- and epoxy-acids. *Ark. Kemi.* **15:** 545–574.
5. De Vries, H. A. M. A. 1970. Polymerization of the cutin acids of the apple skin. *Acta Bot. Neer.* **19:** 41–48.
6. Zimiński, T., and E. Borowski. 1966. A new spray reagent replacing sulphuric acid in thin-layer chromatography. *J. Chromatogr.* **23:** 480–482.