

## Communications to the Editor

[Chem. Pharm. Bull.]  
26(9)5920-2922(1978)

UDC 547.29.02.08 : 543.544.2.061

Occurrence of Odd-Numbered Fatty Acids in *Vibrio cholerae*  
Lipopolysaccharide

Fatty acid components of lipopolysaccharides isolated from *Vibrio cholerae* 35A3 (Inaba) and NIH 90 (Ogawa) have been analysed by gas-liquid chromatography. Considerable amounts of odd-numbered fatty acids, such as nonhydroxy C<sub>15</sub> and C<sub>17</sub> and 3-hydroxy C<sub>11</sub> and C<sub>13</sub> acids, were found particularly in LPS from 35A3 (Inaba) strain.

**Keywords**—lipopolysaccharide; LPS; *Vibrio cholerae*; odd-numbered fatty acid; gas-liquid chromatography; fatty acid composition;

The fatty acid composition of lipopolysaccharides (LPS) of many gram-negative bacteria has been well documented.<sup>1)</sup> The main fatty acids present in LPS isolated from various bacterial sources are usually even-numbered nonhydroxy and 3-hydroxy fatty acids such as C<sub>12</sub>, C<sub>14</sub> and C<sub>16</sub> acids. However, in the case of *Veillonella*,<sup>2)</sup> *Brucella*,<sup>3)</sup> *Myxobacteria*<sup>4)</sup> and *Selemonomonas ruminantium*,<sup>5)</sup> odd-numbered fatty acids such as nonhydroxy and 3-hydroxy C<sub>13</sub>, C<sub>15</sub> and C<sub>17</sub> acids are the major fatty acids in their LPS.

In this study, the fatty acid composition of LPS isolated from the two selected strains of *Vibrio cholerae*, *V. cholerae* 35A3 (Inaba) and NIH 90 (Ogawa) has been investigated by gas-liquid chromatography. Large amounts of nonhydroxy C<sub>14</sub> and C<sub>16</sub> and 3-hydroxy C<sub>12</sub> and C<sub>14</sub> acids were found as the major fatty acids in LPS of the two strains. Unexpectedly, however, considerable amounts of odd-numbered fatty acids, such as nonhydroxy C<sub>15</sub> and C<sub>17</sub> and 3-hydroxy C<sub>11</sub> and C<sub>13</sub> acids, were found particularly in LPS of Inaba type strain.

Microbes were grown in 1% glucose-peptone medium, pH 8.0, at 30° for 16 hr. Cells were treated with 1% phenol for 12 hr at room temperature. Cell walls were prepared according to Hisatsune *et al.*<sup>6)</sup> by shaking the cells in Bühler's cell homogenizer (Edmund Bühler, Tübingen, West Germany) with glass beads (0.11–0.12 mm in diameter). LPS were isolated and purified from either acetone dried or lyophilized crude cell walls essentially by means of Westphal's phenol-water technique<sup>7)</sup> and repeated ultracentrifugation and RNase treatment. Gas-liquid chromatography was performed using An F and M model 7624 A. A glass-column, 2 m × 4 mm, packed with 3% OV-1 coated on Chromosorb W or 25% DEGS on the same support was used with temperature programming from 150° at 1°/min. The identities of the fatty acids were based on retention time values compared to those of authentic standards.

About 3 mg of LPS were hydrolysed with 0.5 ml of 4 N HCl for 4 hr at 100°. The fatty acids released were extracted with ether, and extracts were evaporated to dryness under a flow of nitrogen, yielding "Residue 1" (Total lipid fraction). Fatty acids present in Residue 1 were esterified according to the method of Hoshi *et al.*<sup>8)</sup> in a solution of 0.2 ml of 20 mM methanolic Cu(OAc)<sub>2</sub>, 0.2 ml of CH<sub>3</sub>Cl and 1 ml of 0.5 N methanolic HCl for 1 hr at room temperature. The total lipid content was 31% and 25% and the total fatty acid content, when

- 1) O. Lüderitz, C. Galanos, V. Lehman, M. Nurminen, E.Th. Rietschel, G. Rosenfelder, M. Simon and O. Westphal, *J. Infect. Dis.*, **128**, S 17 (1973).
- 2) M.J. Hewett, K.W. Knox and D.G. Bishop, *Eur. J. Biochem.*, **19**, 169 (1971).
- 3) C. Lacave, J. Asselineau, A. Serre and J. Roux, *Eur. J. Biochem.*, **9**, 189 (1969).
- 4) G. Rosenfelder, O. Lüderitz and O. Westphal, *Eur. J. Biochem.*, **44**, 411 (1974).
- 5) Y. Kamio, K. C. Kim and H. Takahashi, *J. Biochem.* (Tokyo), **70**, 187 (1971).
- 6) K. Hisatsune, T. Kawata, S. Shimodori and K. Takeya, Symposium on Cholera, Pro. 8th Joint Conference, U.S. -Japan cooperative Med. Sci. Program, Cholera Panel, 1972, p. 9.
- 7) L. Westphal, O. Lüderitz and F. Bister, *Z. Naturforsch.*, **76**, 148 (1952).
- 8) M. Hoshi, M. Williams and Y. Kishimoto, *J. Lipid Res.*, **14**, 559 (1973).

recovered as methyl esters, was 18% and 7% of LPS isolated from 35A3 (Inaba) and NIH 90 (Ogawa) strains, respectively. The total fatty acid methyl ester fraction was subjected to preparative thin-layer chromatography (Silica Gel G, hexane-ethyl ether-acetic acid, 70:30:1, V/V/V) in order to isolate nonhydroxy fatty acid methyl esters and 3-hydroxy fatty acid methyl esters. The ratio of the amounts of each esters was estimated to be approximately 1:1 in each strain.

The results of analysis of the nonhydroxy fatty acid fraction by gas-liquid chromatography are as follows (percent values in parentheses represent contents in the total nonhydroxy fatty acid fractions from LPS of 35A3 (Inaba) and NIH 90 (Ogawa) strains, respectively). The major nonhydroxy fatty acid components of LPS of the two strains were found to be  $C_{14:0}$  (27% and 21.5%) and  $C_{16:0}$  (49.1% and 49.4%). In addition, small but significant amounts of  $C_{18:0}$  (0.8% and 3.3%),  $C_{16:1}$  (1.9% and 10.9%) and  $C_{18:1}$  (0.3% and 2.7%) also observed in LPS of the two strains. It is of particular interest that considerable amounts of odd-numbered fatty acids were found particularly in LPS of 35A3 (Inaba) strain: these were  $C_{15:0}$  (12.2%) and  $C_{17:0}$  (6.3%). In contrast, only 1.1% of  $C_{15:0}$  and trace amount of  $C_{17:0}$  were found in LPS of NIH 90 (Ogawa) strain.

With regard to 3-hydroxy fatty acid composition,  $C_{12h:0}$  and  $C_{14h:0}$  were found to be present as the major acids in LPS of both strains. Their contents in the total hydroxy fatty acid fractions from LPS of 35A3 (Inaba) and NIH 90 (Ogawa) strains were 37.0% and 42.9% for  $C_{12h:0}$  and 46.3% and 54.7% for  $C_{14h:0}$ , respectively. Furthermore, substantial amounts of odd-numbered fatty acids were also observed particularly in LPS of 35A3 (Inaba) strain, these being  $C_{11h:0}$  (3.1%) and  $C_{13h:0}$  (13.6%), while only trace amounts of the two acids were found in LPS of NIH 90 (Ogawa) strain.

Of the two O serotypes cholera vibrios, Inaba and Ogawa, only the Inaba type has been investigated with regard to the fatty acid composition of endotoxic LPS. Armstrong and Redmond<sup>9)</sup> and Raziuddin and Kawasaki<sup>10)</sup> reported that, in LPS from both 569B (Inaba) and *El-Tor* (Inaba) strains,  $C_{14:0}$  and  $C_{16:0}$  are the main nonhydroxy fatty acid components, and  $C_{12h:0}$  and  $C_{14h:0}$  are the main 3-hydroxy fatty acid components. They noted that the most abundant 3-hydroxy fatty acid is  $C_{12h:0}$  rather than the more usual  $C_{14h:0}$ . In LPS of 35A3 (Inaba) and NIH 90 (Ogawa), however, quantity of  $C_{14h:0}$  was slightly greater than that of  $C_{12h:0}$ .

It has been known that fatty acid present in LPS of gram-negative bacteria are bound to D-glucosamine disaccharides backbone of lipid A portion through both ester and amide linkages. In general, the amide-linked fatty acid is a 3-hydroxy fatty acid, and if several 3-hydroxy fatty acids occur, the one with the longest chain is amide-linked.<sup>11)</sup> In the present study, we have shown that  $C_{14:0}$ , which is the most usual amide-linked fatty acid in many gram-negative bacteria,<sup>11)</sup> is the longest-chain fatty acid in LPS from the two selected strains of *V. cholerae*, 35A3 (Inaba) and NIH 90 (Ogawa) strains. It would be interesting to know whether 3-hydroxy tetradecanoic acid,  $C_{14h:0}$ , is the amide-linked fatty acid in LPS from the two strains of *V. cholerae*, and whether the odd-numbered 3-hydroxy fatty acid, 3-hydroxy tridecanoic acid,  $C_{13h:0}$ , present in LPS from 35A3 (Inaba) strain, is involved in amide-linkage, just as in LPS from *Veillonella*, where  $C_{13h:0}$  and  $C_{15h:0}$  are amide-linked.<sup>2,12)</sup> These questions are at present under detailed investigation.

**Acknowledgement** We are grateful to Dr. Yasuo Kishimoto (supported in part by NIH research grants NS-13559, NS-13509 from the United States Public Health Service), John F. Kennedy Institute, Johns Hopkins University, School of Medicine, for his collaboration in fatty acid analysis.

9) I.L. Armstrong and J.W. Redmond, *Biochem. Biophys. Acta*, **348**, 302 (1974).

10) S. Raziuddin and T. Kawasaki, *Biochim. Biophys. Acta*, **431**, 116 (1976).

11) E.Th. Rietchel, C. Galanos and L. Lüderitz, in D. Schlessinger, *Microbiology-1975*, American Society for Microbiology, Washington, D.C., 1975, p. 307.

12) D.G. Bishop, M.J. Hewett and K.W. Knox, *Biochem. Biophys. Acta*, **231**, 274 (1971).

Department of Microbiology  
Josai University School of  
Pharmaceutical Sciences  
Sakada, Saitama 350-02, Japan

KAZUHITO HISATSUNE  
SEIICHI KONDO

Department of Food Microbiology  
Tokushima University School  
of Medicine  
Tokushima 770, Japan

TOMIO KAWATA

Received May 8, 1978

[Chem. Pharm. Bull.]  
26(9)2922-2923(1978)

UDC 547.931.08 : 543.544.2.062

### Analysis of Bile Acids and Cholesterol by Open Tubular Glass Capillary-Selected Ion Monitoring

A method for the baseline separation of the dimethylethylsilyl (DMES) ether derivatives of bile acid ethyl esters and cholesterol was developed by using selected ion monitoring (SIM) with open tubular glass capillary column coated with SE-30. The DEMS ether derivatives of bile acid ethyl esters were eluted in the regular order according to the number of hydroxyl group in the molecule. The detection limit of the DMES ether derivative of lithocholic acid ethyl ester was 5 pg with S/N ratio at 5:1.

**Keywords**—bile acids; profile analysis; dimethylethylsilyl ethers; capillary SIM; WCOT column;

In biomedical field, much attention has been focused on the development of an analytical method for metabolic profile of endogenous substances in biological fluids, because it may provide a valuable information to elucidate their physiological role.

Gas chromatography (GC) equipped with open tubular glass capillary column has already been used as a powerful tool for separation of endogenous substances, especially such as steroids<sup>1)</sup> and prostaglandins<sup>2,3)</sup> which have very similar structure one another. The combination of high performance capillary GC and extremely sensitive selected ion monitoring (SIM) has been expected to bring great advantages in the analysis of endogenous substances in biological fluids.

GC separation of bile acids has been performed exclusively on packed column with various stationary liquid phases using various derivatives suitable for enhancing the GC resolution.<sup>4-6)</sup> However, it may be difficult to apply these conventional methods using a packed column to the analysis of metabolic profile of cholesterol bile acid transformation.

The present communication deals with a complete separation method by GC-SIM with wall-coated open tubular glass capillary column (WCOT) for the compounds related to biotransformation from cholesterol to bile acids.

In this study, bile acids (lithocholic acid: LCA, deoxycholic acid: DCA, chenodeoxycholic acid: CDCA, ursodeoxycholic acid: UDCA and cholic acid: CA) were converted to their

- 1) A.L. German, C.D. Pfaffenberger, J-P. Thenot, M.G. Horning and E.C. Horning, *Anal. Chem.*, **45**, 930 (1973).
- 2) J. Maclouf, M. Rigaud, J. Durand and P. Chebroux, *Prostaglandins*, **11**, 999 (1976).
- 3) F.A. Fitzpatrick, *Anal. Chem.*, **50**, 47 (1978).
- 4) P. Eneroth and J. Sjövall, in P.P. Nair and D. Krotchervsky (Eds.), "The Bile Acids," vol. I, Plenum Press, New York, 1971. p. 121,
- 5) K. Imai, Z. Tamura, F. Mashige and T. Osuga, *J. Chromatogr.*, **120**, 181 (1976).
- 6) H. Miyazaki, M. Ishibashi and K. Yamashita, *Biomed. Mass Spectrom.*, in press.