S-methylsulfonium ion intermediate. Isopentenyl pyrophosphate[14] is a key compound in the biosynthesis of long-chain terpenes and, ultimately, steroid hormones. A simple hydrogenation of it would produce isopentyl methyl sulfide.

Finally, we noticed another sex-related difference involving carbonyl compounds in wolf urine. Utilizing isolation and selective derivatization of carbonyl compounds[15] from wolf urine, followed by separation and quantitation by reversed-phase HPLC, samples from male, female, castrate male, and ovariectomized female urines (pooled from November 1981 to February 1982) were compared. The most notable differences between chromatograms were in the peaks identified (through retention times and MS) as hexadecanal and octadecanal. Peak areas reported in arbitrary units were 1.80 and 1.49, respectively, for the normal male, 0.11 and 0.15, respectively, for the normal female, 0.17 and 0.11 for the castrate male, and 0.13 and 0.10 for the ovariectomized female. Thus, these 2 long-chain aldehydes could also play a role in chemical communication in the wolf. Behavioral testing with these compounds is currently being conducted.

While it seems clear[3,5] that the wolf utilizes olfactory means of communication effectively, a possible role of the urinary constituents reported here must now be elucidated. Behavioral tests are currently being designed and carried out for compounds identified and synthesized in this work in either synthetic mixtures or as a part of natural samples.

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0014-4754/84/070707-03$1.50 + 0.20/0

D-3-Dodecanoyltetradecanoic acid as a constituent of lipid A from the lipopolysaccharide of *Yersinia pseudotuberculosis*

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Summary. D-3-Dodecanoyltetradecanoic acid has been separated from the lipid A of *Yersinia pseudotuberculosis* and its structure has been established by chromato-mass-spectrometry and ^{13}C NMR spectroscopy, by comparison with authentic samples.

It was recently shown that an acyloxy fatty acids both ester-bound[1] and amide-linked[2] are constituents of lipid A from the lipopolysaccharides (LPS) of gram-negative bacteria. Together with other fatty acids they are considered to be responsible for the endotoxic properties of lipid A such as pyrogenicity, toxicity, mitogenicity, and others. A mild alkaline treatment of lipid A or LPS decreases or abolishes the aforementioned activities[3] and leads to formation of free fatty acids. The aim of the present work was the isolation of acyloxy fatty acid from the lipid A of *Y. pseudotuberculosis*.

The lipid A was prepared by mild acid hydrolysis of *Y. pseudotuberculosis* LPS (IB serovar 598 strain) with 1% acetic acid[4]. The acyloxy acid together with other fatty acids was obtained by mild alkaline hydrolysis of lipid A (0.25 N NaOH, 56 °C, 15 min)[1]. The fatty acid mixture obtained was separated on the column with silica gel L (40/100 μ, CSSR, 1 × 12 cm) in the system: hexane (20 ml), hexane: diethyl ether (99:1, 20 ml; 95:5, 20 ml; 50:50, 20 ml). As a result dodecanoic ($M^+ = 214$, m/z 199, 183 for methyl ester[5]), D-3-hydroxytetradecanoic ($[\alpha]_D^{20}$ −11.8° (c 0.7, $CHCl_3$)[1]; $M^+ = 258$, m/z 240, 227, 208, 103 for methyl ester[5]), and D-3-dodecanoyltetradecanoic ($M^+ =$ 440, m/z 409, 240, 241, 208, 209 for methyl ester[2]) acids were obtained. Treatment of the latter with sodium methylate yielded dodecanoic and D-3-hydroxytetradecanoic acids in the ratio 1:1. It should be noted that the same mixture of fatty acids is formed during the preparation of the lipid A from LPS by acid hydrolysis and it may also be used for isolation of acyloxy acids. A lightness of the formation of these fatty acids points to their ester linkage with a residue of glucosamine.

^{13}C NMR spectroscopy data on some D,L-3-hydroxytetradecanoic acid derivatives

Atom	Shifts in compound (ppm)							
	I	Ia	II	IIa	III	IV	IVa	lip A
C-2	41.2	41.3	38.9	39.0	39.0	39.0	39.2	38.8
C-3	68.1	68.1	70.6	70.6	70.2	70.0	70.3	70.2
C-4	36.6	36.6	34.0	34.1	34.3	34.5	34.6	34.5
C'-2					34.0	34.1	33.9	34.0
C'-3					24.9	25.0	25.0	24.9
C'-4					29.7	29.7	29.7	29.7
C=O	177.7		174.8	170.1	176.2	177.6	173.1	174.0
C'=O		173.5	170.7	170.7	173.4	172.7	170.8	170.6

Finally, the structure of *Y. pseudotuberculosis* acyloxy acid was confirmed by ^{13}C NMR spectroscopy. For interpretation of the spectroscopic data, D,L-3-hydroxy-(I)[6], D,L-3-acetoxy-(II)[7], D,L-3-dodecanoyl-(III), and D,L-3-tetradecanoyltetradecanoic-(IV) acids were synthesized and ^{13}C NMR spectra of these compounds and their methyl esters (Ia–IVa) were studied. To prepare D,L-3-tetradecanoyltetradecanoic acid (IV), tetradecanoyl chloride (10 µmoles) was added to a solution of the compound I (5.5 µmoles) and p-dimethylpyridine (0.5 µmoles) in dry chloroform (40 ml) and dry pyridine (10 ml) at −10 °C. After 20 h at room temperature the reaction mixture was poured into ice-water, the organic layer was washed with 1 N HCl and evaporated. The residue was separated by sephadex LH-20 column chromatography with chloroform as eluent to yield the compound IV (melting point 38.5 °C, 54%). The compound III was obtained in an analogous way (oil, 52%). The fatty acids methyl esters were prepared by treatment with diazomethane.

Compounds: I, D,L-3-hydroxy-; II, D,L-acetoxy-; III, D,L-3-dodecanoyl-; IV, D,L-3-tetradecanoyltetradecanoic acids; Ia–IVa, methyl esters of the aforementioned acids; lip A, acyloxy fatty acid from lipid A of *Y. pseudotuberculosis*. C′-Carbon atoms of acid residue acylating C-3 atom of hydroxytetradecanoic acid. The chemical shift values of other carbon atoms: C-5–C-11 = 29.7; C-12 = 32.0; C-13 = 22.7; C-14 = 14.1; OCH_3 = 51.7 ppm.

An analysis of the data in the table shows that acylation of the hydroxyl group on the C-3 atom of 3-hydroxytetradecanioc acid shifts the signals of C-2 (up field), C-3 (down field), and C-4 (up field) atoms. It seems possible to use these finding of the structure investigation of the native lipid A as well. Thus, the results of ^{13}C NMR spectroscopy, chromato-mass-spectrometry, and alkaline degradation point to the presence of dodecanoyltetradecanoic acid in the lipid A of *Y. pseudotuberculosis*. Synthesis of glucosamine derivatives with hydroxy- and acyloxy fatty acids may help to elucidate the role of the latter in the biological activity of lipid A.

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0014-4754/84/070709-02$1.50 + 0.20/0

Effect of hormones and cyclic AMP on γ-glutamyltranspeptidase activity of rat mammary gland explants[1]

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Summary. γ-Glutamyl transpeptidase activity was assayed in midpregnant rat mammary gland explants at 14, 22 and 38 h, in the presence and absence of insulin, prolactin and corticosterone. With these 3 hormones the explants attained the characteristics of a secretory gland after 22 h of tissue culture, at which time the enzyme exhibited its maximal activity. The addition of dibutyryl cyclic AMP in the presence of the 3 hormones produced a significant increase in enzyme activity, which was maximal with a 1 mM concentration of the cyclic nucleotide. A similar effect was observed when theophylline or theophylline plus dibutyryl cyclic AMP were added to the culture medium.

γ-Glutamyl transpeptidase is a membrane enzyme which has been described in several tissues, specially those which have a secretory function[2]. The metabolic turnover of reduced glutathione (GSH) is related to transpeptidase activity since this enzyme removes the γ-glutamyl moiety of the tripeptide, catalysing one of the first steps of a detoxification pathway which leads to mercapturic acid formation[3]. Another important function attributed to γ-glutamyl transpeptidase is related to the incorporation of amino acids into the cell through six enzymatic reactions known as the γ-glutamyl cycle[4]. In a previous publication we described the transpeptidase activity in the mammary gland of the rat during the lactogenic cycle, where we found a maximum of activity at the tenth day of lactation[5]. We have also reported some properties of this enzyme from rat mammary gland[6], as well as the presence of three enzymes of the γ-glutamyl cycle whose activities increased during lactation[7]. Here, we present a study of γ-glutamyl transpeptidase activity in rat mammary explants and the effect of insulin, prolactin and corticosterone and of cyclic adenosine monophosphate (cAMP) on this enzyme activity.

Material and methods. L-γ-glutamyl-p-nitroanilide, glycylglycine, prolactin, corticosterone, theophylline, dibutyryl cyclic AMP and cycloheximide were obtained from Sigma Chemical Co.; insulin from Lilly Laboratories and Eagle's Medium Modified by Dulbecco (MEMD), penicillin and streptomycin from Flow Laboratories. Mammary gland explants (2–3 mg each) from Sprague-Dawley midpregnant rats (12–13 days of pregnancy) were incubated in MEMD with glutamine (0.584 mg/ml), sodium bicarbonate (1.25 mg/ml) and penicillin and streptomycin (50 U/ml each). The hormones used in this study and their concentrations in the culture media were as follows: ovine prolactin (33 IU/mg) 10 µg/ml; bovine pancreatic insulin (23 IU/mg) 5 µg/ml and corticosterone 1 µg/ml[8].

The explants were removed and homogenized in 4 vol. 0.15 M Tris (pH 8.0)/0.01 M KCl in an Ultra-Turrax blendor (Janke and Kunkel, Staufen). The enzymatic assay was carried out using whole homogenates with L-γ-glutamyl-p-nitroanilide as donor and glycylglycine as acceptor[9]. Enzymatic activity is expressed as µmoles of product formed per minute at 37 °C (units) per mg of protein. Lactose was assayed enzymatically with β-galactosidase and β-galactose dehydrogenase[10]. Proteins were determined by Lowry's method[11].

Results. The time course of γ-glutamyl transpeptidase activity in mammary gland explants incubated with insulin, prolactin