Review

The biological significance of ω-oxidation of fatty acids

By Yoshiro MIURA[*1,†]

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Abstract: The author focuses on the biological significance of ω-oxidation of fatty acids. Early studies revealed that there is a subsidiary pathway for β-oxidation of fatty acids when β-oxidation is blocked. Many studies demonstrated that the ω-oxidation serves to provide succinyl-CoA for the citric acid cycle and for gluconeogenesis under conditions of starvation and diabetes. Acylglucosylceramides which are composed of linoleic acid, long chain ω-hydroxy fatty acids, eicosasphingenine (or trihydroxyeicosasphingenine) and glucose, are responsible for normal epidermal permeability function in the skin. It is observed that ω- and (ω-1)-oxidation of fatty acids are related to energy metabolism in some laboratory animals such as musk shrews and Mongolian gerbils. Studies confirmed that ω- and (ω-1)-oxidation of fatty acids play crucial roles in the production of insect pheromones of honeybees and in the formation of biopolyesters of higher plants. In addition, the biological significance of ω-oxidation of prostaglandins and leukotrienes is described.

Keywords: ω-oxidation, (ω-1)-oxidation, fatty acid, cytochrome P-450, biological significance, energy metabolism

Introduction

Fatty acids are important not only because of their role as a high energy source but also because they serve as essential constituents of natural lipids such as cholesterol ester, phospholipid, and glycolipid which are major components of biomembranes. Several pathways for the oxidation of fatty acid have been observed in living organisms. The most important and well-known pathway for fatty acid oxidation is β-oxidation, in which fatty acids are converted into acetyl Co-A or propionyl Co-A (derived from odd-numbered fatty acid chains), and finally those Co-As are oxidized into carbon dioxide and H_2O resulting in energy production.

α-Oxidation, the removal of one carbon at a time from the carboxyl end of fatty acid chain, has been reported in yeast,[1)] plants,[2)] and mammal.[3)] Although α-oxidation does not require CoA intermediates (*i.e.*, acyl-CoAs) and does not generate a high-energy phosphate, α-oxidation is important in the metabolism of phytanic acid. Since phytanic acid contains a methyl group on the 3-carbon position, β-oxidation of the acid is blocked, but the acid can be degraded by β-oxidation after the removal of one carbon from carboxylic end of the acid by α-oxidation (Fig. 1). In patients with Refsum's disease, α-hydroxylase which catalyzes the first degradation step of phytanic acid is missing or has low activity.[4)] Consequently, phytanic acid accumulates in patients with Refsum's disease. Such patients may present with symptoms such as peripheral nerve dysfunction, muscle paralysis and visual field defects as a result of this accumulation.

Verkade *et al.*[5)] first proposed ω-oxidation of fatty acids *in vivo*. They showed that considerable amounts of dicarboxylic acids were excreted in the urine, when medium and long chain fatty acids were administered to animals or humans.[5),6)] In a subsequent study, Verkade[7)] showed that ω-oxidation of fatty acids involved two reaction steps (Fig. 2). First, the terminal $-CH_3$ group of fatty acids is converted to a $-CH_2OH$ group, and subsequently it is oxidized to a $-COOH$ group. Formed dicarboxylic acids are β-oxidized usually from both ends of the fatty acid chain to shorter dicarboxylic acids (bilateral β-oxidation). Although acyl-CoAs are required for

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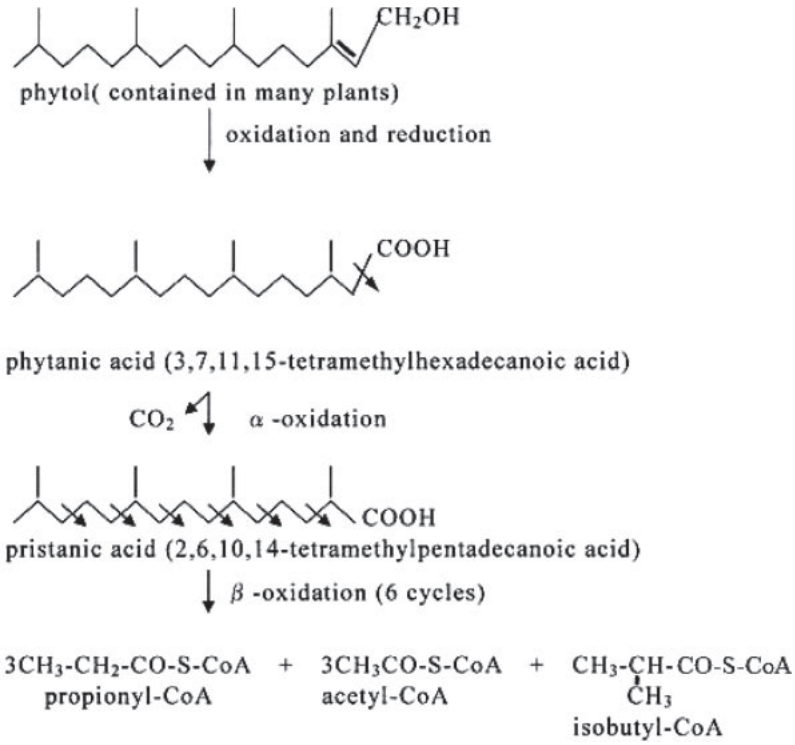

Fig. 1. Degradation (α- and β-oxidation) of phytanic acid.

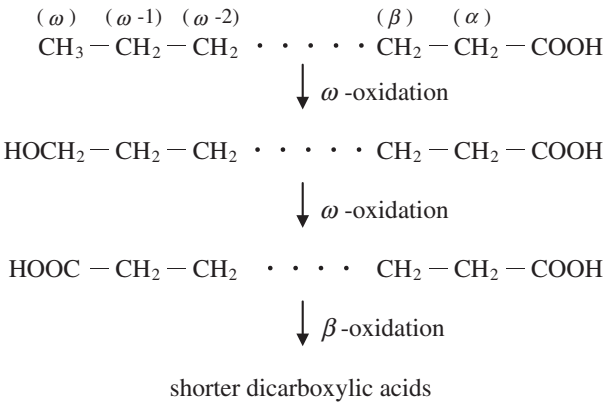

Fig. 2. Reaction step of ω-oxidation of fatty acid.

metabolic pathways such as β-oxidation and biosynthesis of triacylglycerols, ω-oxidation does not require acyl-CoAs as shown in Fig. 2.

The *in vitro* ω-oxidation of short and medium chain fatty acids was reported by Wakabayashi and Shimazono.[8] They suggested that a mixed function oxidase is involved in the ω-oxidation. Robbins[9] also reported that the first step (ω-hydroxylation) is carried out by the microsomal fraction of pig, dog and rat liver. Although the characteristics of the enzyme(s) which catalyzed the ω-oxidation are not elucidated in great detail by these investigators, Lu and Coon[10] clearly demonstrated that hepatic microsomal cytochrome P-450 and NADPH-cytochrome *c* reductase are responsible in the ω-oxidation of lauric acid. In the same year, Wada *et al.*[11] also demonstrated the involvement of cytochrome P-450 and NADPH-cytochrome *c* reductase in the ω-oxidation of stearic acid. Since then, many investigators have reported that the enzyme systems involving the microsomal or mitochondrial cytochrome P-450 catalyzed the ω-oxidation of various fatty acids, and it has been established that cytochrome P-450 (a heme iron protein) plays an important role in the ω-oxidation of fatty acids. It was shown in recent studies that the cytochrome P-450 which catalyzes the ω-oxidation of fatty acids belongs to CYP4A family.[12] On the other hand, Reuttinger *et al.*[13] reported that a non-heme iron protein catalyzes the ω-hydroxylation of fatty acids in *Pseudomonas oleovolans*.

Ichihara *et al.*[14] reported on the distribution of ω-oxidation of fatty acids: The ω-hydroxylation of decanoic and lauric acid is carried out by liver, kidney and lung microsomal preparations of rat, rabbit, mouse, beef, pigeon, bullfrog, and carp (Table 1).

Table 1. ω-Hydroxylation activity of decanoic and lauric acid by microsomal preparation of tissues of various species[14]

Species	ω-hydroxylation activity (nmole/30 min per mg protein)					
	Liver		Kidney		Lung	
	C_{10}	C_{12}	C_{10}	C_{12}	C_{10}	C_{12}
Rat	4.48	8.40	9.33	7.22	0.00	0.00
Rabbit	—	25.22	—	13.38	—	22.00
Mouse	12.60	3.74	—	6.92	—	6.06
Beef	3.16	4.55	—	—	9.52	7.04
Pigeon	0.25	1.46	2.04	2.93	0.00	0.00
Bullfrog	—	19.82	—	—	—	—
Carp*	—	11.90	—	—	—	—

Abbreviations: C_{10}, decanoic acid: C_{12}, lauric acid (dodecanoic acid)
*The 10,000 × g supernatant was used for assay.

Miura *et al.*[15)–20)] described in detail some properties of the fatty acid ω-hydroxylating system in the amphibia. Since ω-oxidation of fatty acids are also found in the fungi,[21)] the higher plants[22)] and the insects,[23)] it is suggested that ω-oxidation could be universally found in nature. Moreover, ω-type oxidations such as (ω-1)-, (ω-2)- and (ω-3)-oxidation have been found in variety of living organisms.[24)–27)] Hydroxylase which is found in *Bacillus megaterium*[24),25)] catalyzing mainly (ω-2)-hydroxylation of fatty acids is named as P-450BM3 (CYP102A1).[28)] However, detailed information is not available on the biological significance of the ω- and ω-type oxidation of fatty acids. In this review, the author describes several plausible biological roles of the ω- and ω-type oxidations of fatty acids in animals including humans as well as plants.

1. Biological significance of the ω-oxidation of fatty acids

(1) ω-Oxidation is a subsidiary oxidation pathway of β-oxidation. Yamakawa[29)] reported that 2-propyladipic acid is excreted in the rabbit urine, when 2-propylmyristic acid is administered. He proposed that 2-propylmyristic acid is oxidized from ω-methyl terminal by ω-oxidation because the substitution at the 2(α)-carbon atom hinders the usual β-oxidation: the resulting dicarboxylic acid is β-oxidized (unilateral oxidation) from the unsubstituted end 4 times leaving the substituted adipic acid. Weitzel[30)] also reported that 2-butyladipic acid is excreted in dog urine, when 2-butylstearic acid is administered. Bergstrom *et al.*[31)] demonstrated that 2,2-dimethysuccinic acid and 2,2-dimethyladipic acid are excreted in rat urine, when 2,2-dimethylstearic acid, which is disubstituted at the 2(α)-carbon atom, is given (Fig. 3). Therefore, these studies show that ω-oxidation is a subsidiary oxidation pathway of β-oxidation for the fatty acids when the β-oxidation is blocked.

(2) Antiketogenic effect and gluconeogenesis of dicarboxylic acids in diabetes or starvation. Verkade[7)] proposed that dicarboxylic acids which are formed by ω-oxidation, are more efficiently oxidized through β-oxidation than monocarboxylic acids under normal conditions. However, Flaschentrager and Bernhard[32)] reported that contribution of ω-oxidation of fatty acids is less significant. Although little information on the biological significance of ω-oxidation of fatty acids was available at the time of these older reports, in 1973–1980 many groups reported on the oxidation under condition of starvation or diabetes. Bjorkhem[33)] reported that ω-oxidation of stearic acid was significantly increased by 20,000 × g supernatant fluid of rat liver homogenate after starvation or alloxan-induced diabetes. Subsequently, Bjorkhem[34)] demonstrated that the concentration of adenosine-5′-triphosphate (ATP) in the 20,000 × g supernatant fluid of starved rat liver homogenate affected the degree in ω-oxidation of stearic acid. The ω-oxidation was increased at the low concentrations of ATP in the supernatant, but the addition of ATP into the supernatant decreased ω-oxidation. These results suggest that a competition exists between the two pathways (ω-oxidation and glycerides formation) of stearic acid. Bjorkhem[35)] also reported that at least a small fraction of fatty acids may be subject to primary ω-oxidation prior to β-oxidation in the ketonic state. On the other hand, Wada and Usami[36)] observed that the incorporation of blood glucose was greater from dicarboxylic acids than from monocarboxylic acids. Also ω-oxidation may be important for production of succinyl-CoA from fatty acids in starved or diabetic rats (Fig. 4).[36)] They calculated that about 15% of palmitic acid were subjected to ω-oxidation and then β-oxidation.[36)] Moreover, they demonstrated that the administration of dicarboxylic acids to starved rats decreased the concentration of ketone bodies in the blood.[36)] Gregersen *et al.*[37)] observed that the substantial ω-oxidation activity of fatty acids in starving newborn infants served to provide succinyl-CoA-substrate for the citric acid cycle and as well as for gluconeogenesis. Mortensen[38)] demonstrated that the ω-oxidation of fatty acids might have importance metabolic influence in situations where the living

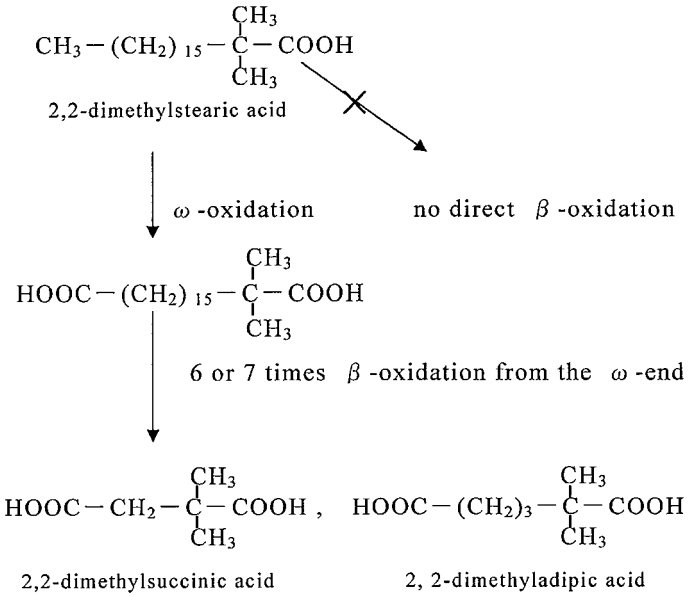

Fig. 3. Degradation of 2,2-dimethylstearic acid in rat.[31]

organisms lacked carbohydrates and largely have to utilize fats for energy demand. Although these reports showed the antiketogenic effect and gluconeogenesis of ω-oxidation of fatty acids, Kam *et al.*[39] reported that ω-oxidation of fatty acids could not contribute more than a small extent to the formation of glucose. Moreover, glucose was not formed via ω-oxidation of long-chain fatty acid.[40] Thus the antiketogenic effect and gluconeogenicity of ω-oxidation of fatty acids under starvation or diabetic condition are still being debated by these investigators.

(3) Energy production for novel laboratory animals. Although mammals such as rats, mice, guinea pigs or rabbits are generally used in both basic and clinical research, there are numerous species of mammals other than these in nature that, for a variety of specific research needs, may be more suitable as research subjects. Since musk shrew and Mongolian gerbil have been successfully reared and bred,[41)–43)] they have been used for a variety of studies.[44)–47)] These animals have the added desirable traits of small size, short breeding cycles and environmental adaptability. Harvest mice and triton hamsters have also received attention as possible laboratory species for the same reasons.[43),48)] Since little information is available on the properties of their cytochrome P-450-dependent fatty acid hydroxylase systems in these laboratory animals, Miura *et al.* investigated the substrate specificity and other properties of the fatty acid hydroxylase systems in the liver microsomes of the house musk shrews,[49),50)] the Mongolian gerbils,[51)] the Japanese harvest mice[52)] and the triton hamsters.[53)] They found that total hydroxylation activity (*i.e.*, the sum of ω- and (ω-1)-hydroxylation activity) of lauric acids was very high in each of these four animals[53),54)] as compared to the activity in rats (Table 2). In order to elucidate the biological significance of the results, Miura *et al.*[55)] examined the relationship between total hydroxylation activity of lauric acid in the liver of these four animals and their body weight. Significantly, a correlation (correlation coefficient, $r = 0.950$) was obtained between total hydroxylation activity of lauric acid in the livers of these animals and their body weight (Fig. 5).[55)]

The equation relating the body weight (X) to total hydroxylation activity (Y) is as follows: $\log Y = 0.763 + 0.651 \log X$. Similarly, significant correlation ($r = 0.986$, equation: $\log Y = 0.563 + 0.772 \log X$) was observed for total hydroxylation activity of tridecanoic acid in the liver of these animals. The general form, $Y = aX^b$ or $\log Y = \log a + b \log X$ is known as the allometric equation. In this equation Y is a physiological or morphological variable: X is body weight (g, or kg): a and b (usually $b < 1$) are constants.[56)–58)] The values of constant b (0.734 for basal O_2 consumption; 0.77 for O_2 consumption (liver slices); 0.73 for heat production; 0.73 for oxygen flow,

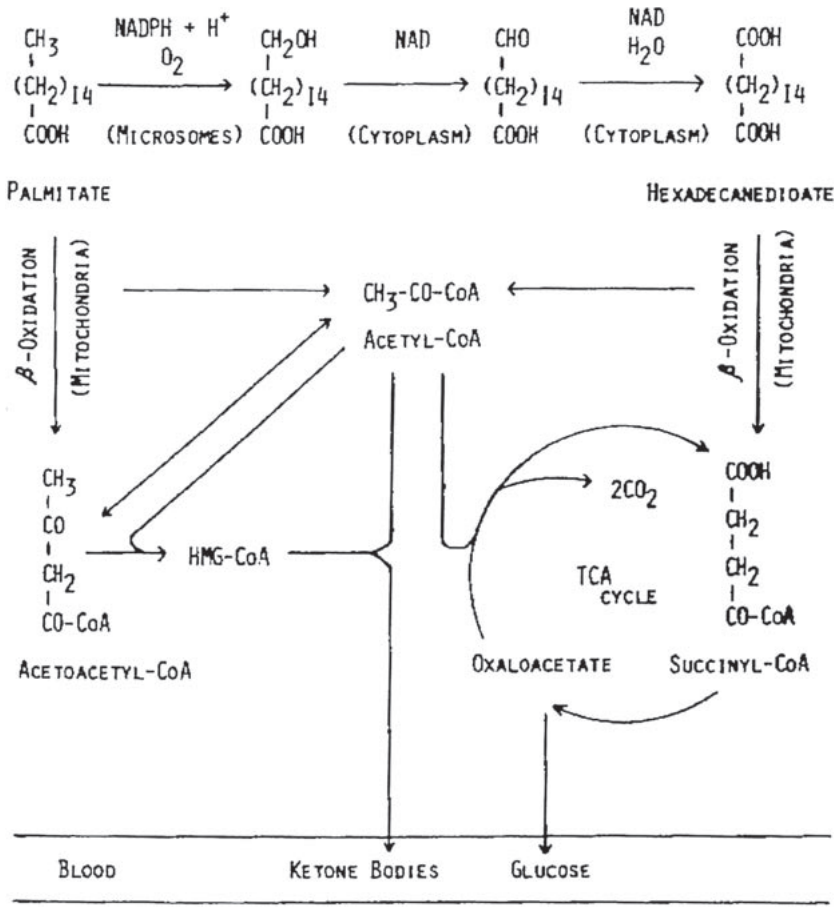

Fig. 4. Metabolic fates of palmitate and hexadecanedioate.[36] NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); NAD, nicotinamide adenine dinucleotide (oxidized form); HMG-CoA, hydroxymethylglutaryl-CoA.

Table 2. Comparison of total hydroxylation activity (sum of ω- and (ω-1)-hydroxylation activities) of saturated fatty acids by liver microsomes of laboratory animals[53),54)]

Chain length	Hydroxylation activity (nmol/mg microsomal protein/min)				
	Shrew	Gerbil	Harvest mouse	Triton hamster	Rat
10	6.53(119)	2.59(47)	10.80(197)	8.84(161)	5.48(100)
12	7.27(212)	5.74(167)	6.87(200)	8.50(248)	3.43(100)
13	5.27(109)	4.59(95)	5.40(111)	8.48(175)	4.85(100)
14	6.34(147)	4.71(110)	9.04(210)	7.74(180)	4.30(100)
16	1.70(69)	3.04(124)	3.61(147)	2.81(114)	2.46(100)
18	0.82(98)	1.15(137)	0.82(98)	0.28(33)	0.84(100)

Values in parentheses are the percentages of each hydroxylation activity of fatty acids by rat liver microsomes.

etc.) for the hepatic and physiological allometric equations[56)–58)] are similar to our values (0.651 for lauric acid; 0.772 for tridecanoic acid) in the equation relating body weight (X) of the laboratory animals to total hydroxylation activity (Y) of lauric acid or tridecanoic acid in liver. The similarity of the b constants suggests a close correlation between the rate of ω- and (ω-1)-hydroxylation and liver energy metabolism in the laboratory animals. In the preceding section, the biological significance of ω-oxidation of fatty acids was discussed in the terms of starvation or diabetes. Since neither starved nor diabetic animals were used by Miura *et al.*, the biological significance of ω- and (ω-1)-oxidation of fatty acids may be generalized to laboratory animals under normal conditions as well.

(4) Skin-specific glycolipids: Epidermoside. Skin is consisted of three layers, epidermis, dermis and subcutaneous tissue, and protects the body from dryness, ultraviolet ray, the invasion of microbes, and chemical and mechanical damages.[59)] The epidermis is the outer layer and its physiological function is the formation of water barrier. Biochemical studies on

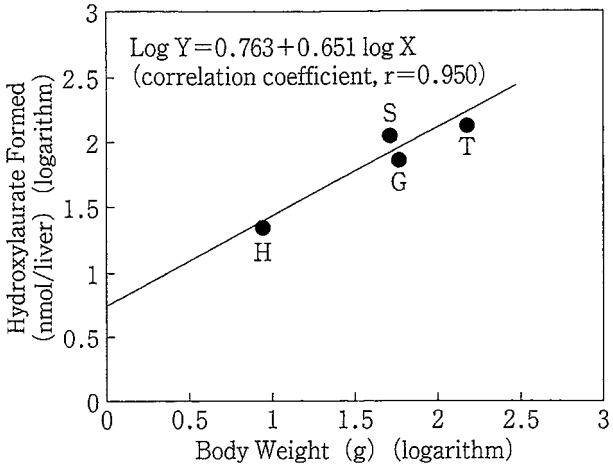

Fig. 5. Relation between body weight (X) of laboratory animals and total laurate hydroxylation activity (Y) in the liver.[55] G, Gerbil; H, Harvest mouse; S, Shrew; T, Triton hamster.

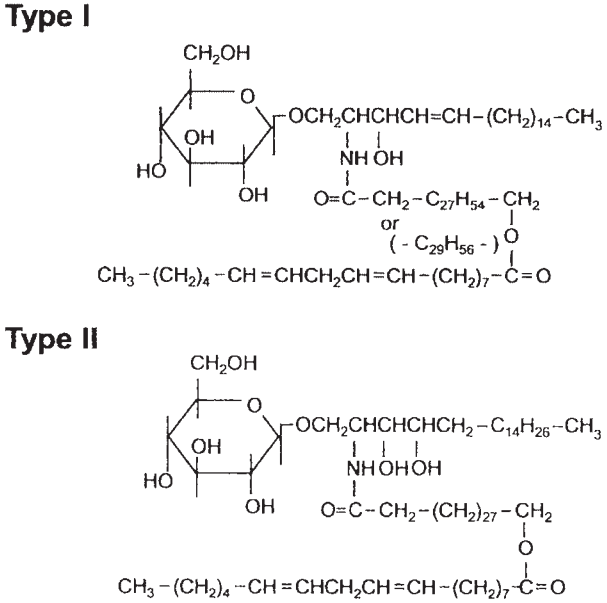

Fig. 6. Structures of human epidermal acylglucosylceramides (Epidermosides).[62]

the structure and the function of epidermis revealed that the acylglucosylceramide (AGC), containing unsaturated fatty acids and very long chain ω-hydroxy fatty acids plays an important role in the formation of water barrier in the epidermis.[60),61)] Hamanaka *et al.*[62)] reported that two types of AGC (Type I AGC and Type II AGC) were contained in human epidermis. They named these AGCs "Epidermosides" (Fig. 6). As shown in Fig. 6, the amide-linked fatty acids of the AGCs is very long chain fatty acid (C_{30}-fatty acid) and linoleic acid is esterified to ω-hydroxy group of C_{30}-fatty acid. Behne *et al.*[63)] reported that omega-hydroxyceramides (ω-OHCers) are required for normal epidermal permeability barrier function and cytochrome P-450 (CYP4A) in epidermis is concerned in the ω-hydroxylation of long chain fatty acids. Thus, ω-hydroxylation of fatty acids in epidermis is crucial to the formation of ω-OHCers.

(5) Role of ω-oxidation of fatty acids in insects and plants. The biological significance of ω- and (ω-1)-type oxidation of fatty acids has been reported in insects and plants. Lercker *et al.*[64)] reported that ω-hydroxy fatty acids were the most abundant components in royal jelly which is essential for biochemical and physiological importance of this substance. Royal jelly is produced by worker bees and fed to queen bees.[64)] Thus royal jelly is the exclusive food of queen bees; the entire life cycle of bees depends upon royal jelly. Queen bees have longevity of 4–5 years, while worker bees live about 2–6 months. Therefore, ω-hydroxy fatty acids may have some nutritional effects related to the longevity of queen bees. Since royal jelly is secreted from the hypopharyngeal and mandibular glands of worker bees, it is assumed that ω-hydroxy fatty acids in royal jelly may be synthesized by cytochrome P-450-dependent enzyme system of worker bees.

Plettner *et al.*[65)] described some interesting functions of ω- and (ω-1)-hydroxy fatty acids which were biosynthesized in the mandibular glands of honeybees (*Apis mellifera* L.) There is a separation of female colony members into reproductive and non-reproductive castes, namely queens and workers bees.[65)] Queens produce 9-hydroxy-(E)2-decenoic acid (9-HDA, or (ω-1)-HDA) and other fatty acids functionalized at the (ω-1)-position and 9-keto-(E)2-decenoic acid (ODA, the oxidation product of 9-HDA). On the other hand, workers produce 10-hydroxy-(E)2-decenoic acid (10-HDA, or ω-HDA) and the corresponding diacids. The investigators proposed the biosynthetic routes of 9-HDA, ODA, 10-HDA and (E)2-decenedioic acid (C10:1 DA) (Fig. 7).[65)] After stearic acid was hydroxylated at the ω- and (ω-1)-positions, the hydroxylated acids were shortened by β-oxidation, and subsequently 9-HDA and 10-HDA were produced. They demonstrated that the queens' acids, that is, 9-HDA and ODA were components of the queen mandibular pheromones which attracted workers to the queens. The reproductive dominance of queens was controlled by the queens' acids. These acids supressed

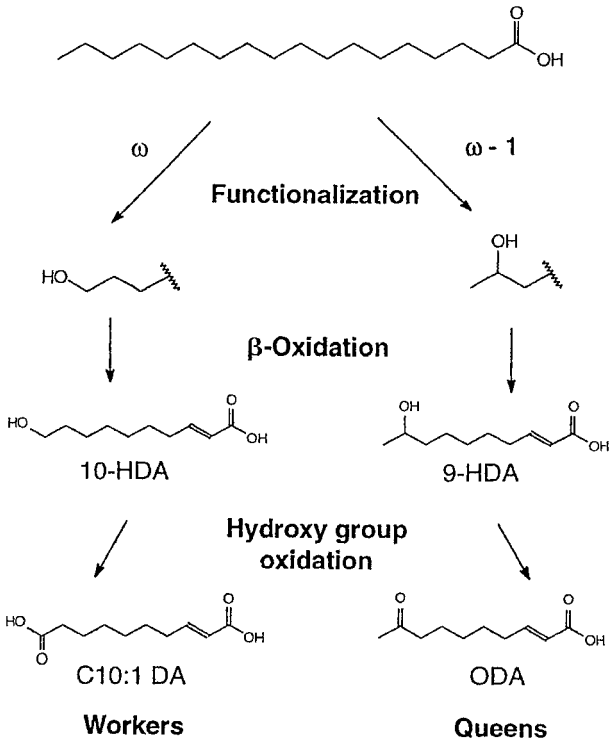

Fig. 7. Biosynthetic route of ω- and (ω-1)-hydroxyl and keto fatty acids from stearic acid in worker and queen honeybees.[65] 10-HDA, 10(ω)-hydroxy-(E)2-decenoic acid; 9-HDA, 9(ω-1)-hydroxy-(E)2-decenoic acid; C10:1DA, (E)2-decenedioic acid; ODA, 9-keto-(E)2-decenoic acid.

the function of the ovary of the workers rendering them infertile. On the other hand, 10-HDA and C10:1DA which were biosynthesized in the mandibular gland of workers did not possess this biological property. Rather these secretions acted as larval nutrients and food preservatives.[65),66)] Thus ω- and (ω-1)-hydroxy fatty acids play an important role in the social and biological lives of honeybees. More recently, Kamakura[67)] reported that royalactin contained in royal jelly induced queen bee differentiation in honeybees. He demonstrated that royalactin induced queen development via an epidermal growth factor receptor (Egfr)-mediated signal pathway. Although he did not describe the correlation between the biosynthesis of 9-HDA and ODA and the function of royalactin, it is estimated that Egfr-mediated signal transduction produced 9-HDA and ODA as a second messenger.

Kolattukudy[68)] reviewed an interesting function of ω-hydroxy, epoxy, and dicarboxylic acids which were contained in polyester compounds in higher plants (Fig. 8). Cutin, a biopolyester composed of ω-hydroxy, epoxy, and dicarboxylic acids, is the barrier between the aerial parts of higher plants and their superterranean environments. Suberin is a polymer in the underground parts and at wounded areas of such plants. Cutin and suberin are composed of two families (a C_{16} family and a C_{18} family) of ω-hydroxy, epoxy and dicarboxylic acids. Therefore, ω-hydroxy fatty acids are essential components of cutin and suberin in higher plants. Kolattukudy and Walton[69)] proposed that two different hydroxylases are involved in the biosynthesis of ω-hydroxy and epoxy fatty acids in cutin and suberin. One of the hydroxylases may be cytochrome P-450. Thus, the biological significance of ω-oxidation of fatty acids was clearly confirmed in higher plants.

(6) Regulation of biological activity of (e)icosanoids. Prostaglandins (PGs) are a derivative of prostanoic acid. Various types of PGs are found in mammalian tissues and organs (Fig. 9).[70)] PGs possess physiologically important activities such as reducing blood pressure, causing smooth muscle contraction, and inducing sleep. PGs are usually biosynthesized from C_{20} unsaturated fatty acids such as dihomo-γ-linolenic acid, arachidonic acid and (e)icosapentaenoic acid. The arachidonic acid-derived PGs are common among PGs. PGs also may be also hydroxylated at ω- and (ω-1) position: These hydroxylated products are further metabolized by β-oxidation, reduction of double bond and oxidation of C15-hydroxy group. The shortened metabolites finally may be secreted in urine. Powell[71)] reported interesting studies on ω-hydroxylation of $PGF_{2\alpha}$ which could contract smooth muscle such as uterus muscle. He observed that ω-hydroxylation $PGF_{2\alpha}$ in rabbit lung and liver microsomes increased about 20-fold on the 25th day of pregnancy. He observed also that progesterone treatment elevated ω-hydroxylation of $PGF_{2\alpha}$ in the rabbits by about 2.4 fold. He suggested that ω-hydroxylation of $PGF_{2\alpha}$ may contribute to maintaining pregnancy. These results showed that the ω-hydroxylation of $PGF_{2\alpha}$ is closely connected with the regulation of biological activity of $PGF_{2\alpha}$. Yamamoto *et al.*[72)] described the biological properties of the cytochrome P-450 (P-450-p-2) which could ω-hydroxylate PGs such as PGE_1, PGE_2, and $PGF_{2\alpha}$ in rabbit lung microsomes. The ω-hydroxylase activity of P-450-p-2 was very low in lung of rabbit, in which progesterone was not administered. However, the ω-hydroxylase activity increased significantly by the administration of progesterone as it does during pregnancy; it diminished quickly after delivery. PGs have well docu-

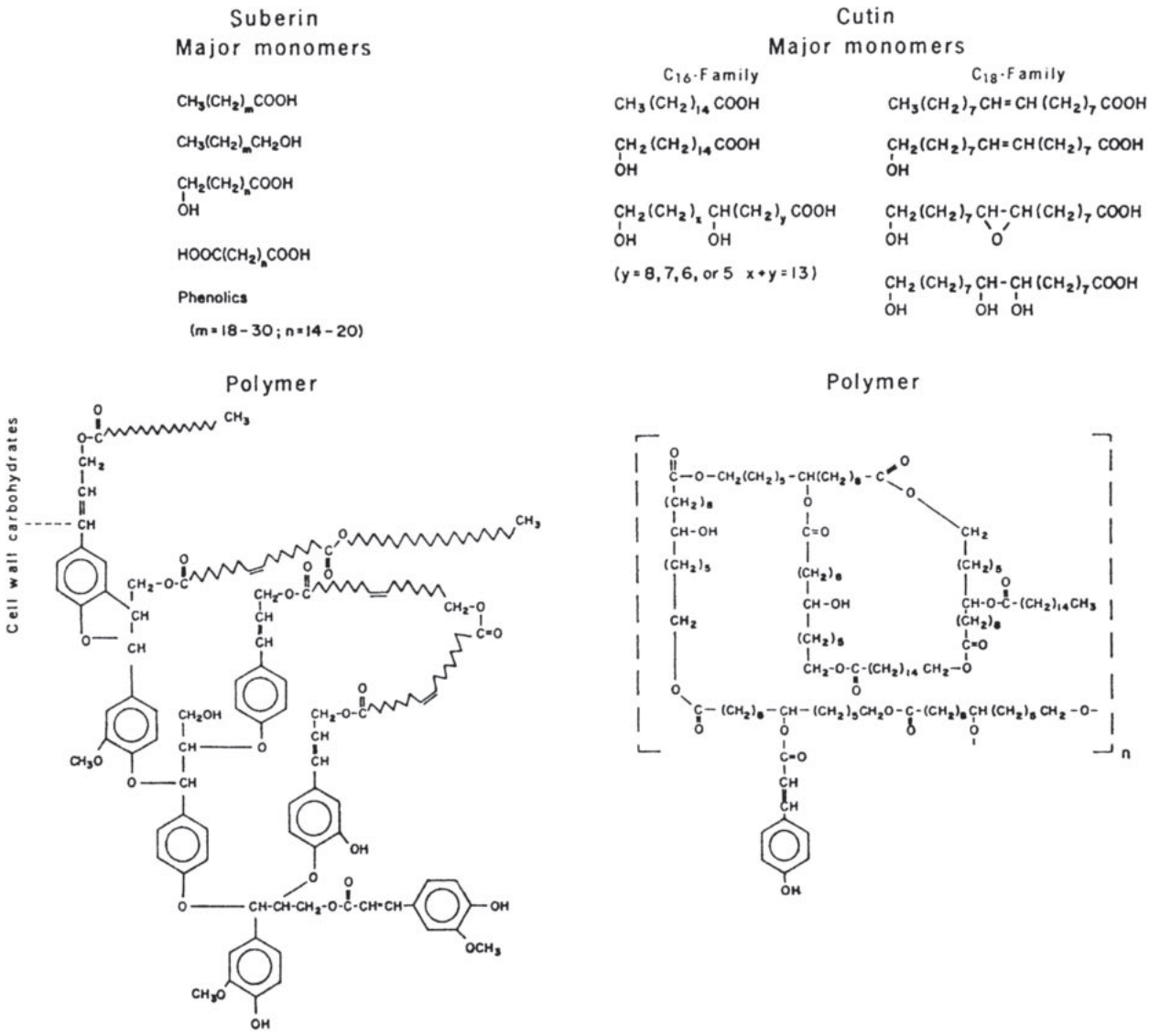

Fig. 8. Structure of monomers of cutin and suberin.[68]

mented physiological and pharmacological activities. On the other hand, ω-hydroxylated PGs lose these activities.[73] Thus the products of ω-hydroxylation of PGs may have some regulatory significance.

Leukotrienes (LTs) are a derivative of polyunsaturated fatty acids. They possess various physiological and pharmacological properties related to immediate hypersensitivity responses and inflammation. It is well known that LTs play an important role in allergic reactions as the slow-reaction-substance of anaphylaxis (SRS-A). LTs, as well as PGs, can be ω-hydroxylated: ω-Hydroxy LTs usually lose their biological activities. Hansson *et al.*[74] reported that in addition to LTB_4, ω-hydroxylated LTB_4 and ω-carboxylated LTB_4 were produced in neutrophils (Fig. 10). The biological activities of LTB_4 metabolites were about 50-times less potent than that of LTB_4. They suggested that the ω-hydroxylation of LTB_4, which is the first step of the inactivation reaction, plays an important role in the control of inflammation. Shak *et al.*[75] and Sumimoto *et al.*[76] revealed that the ω-hydroxylation of LTB_4 was catalyzed at a very low concentration (0.3–0.6 μM) by cytochrome P-450. They named its enzyme LTB_4 ω-hydroxylase. Thus it was confirmed that the ω-hydroxylation of PGs and LTs was closely related to the regulation of the biological activities of these (e)icosanoids.

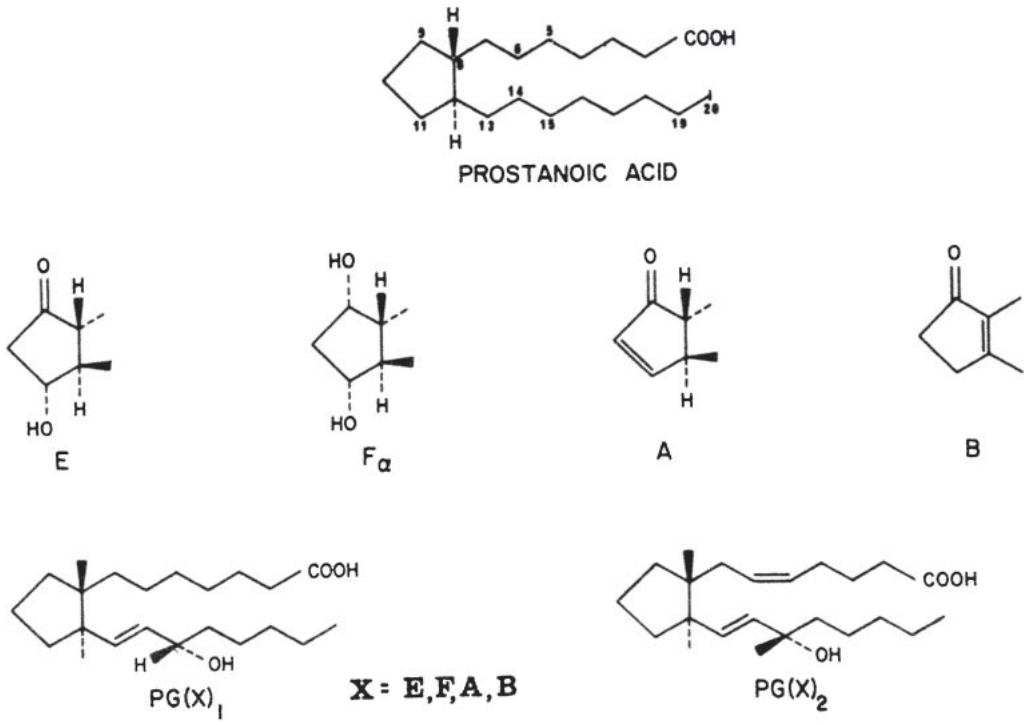

Fig. 9. Structure of prostaglandins (PG, prostanoic acid and derivatives).[70]

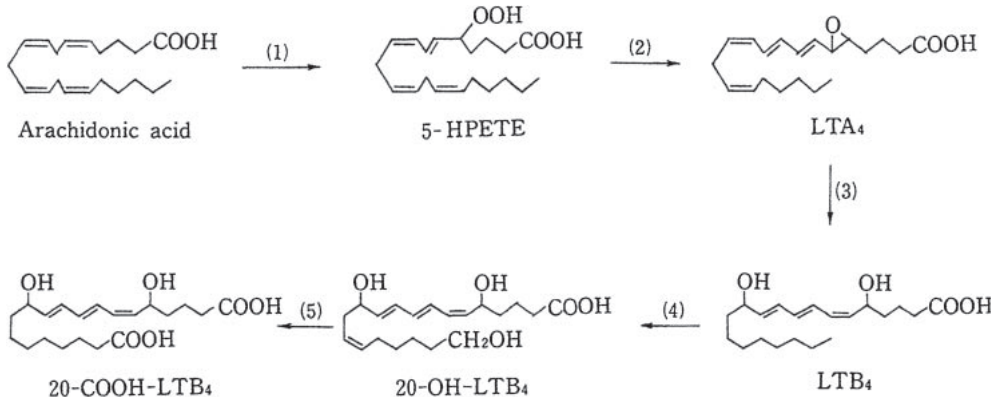

Fig. 10. Biosynthesis and ω-oxidation of leukotriene B_4(LTB_4).[73),74)] (1), 5-lipoxygenase; (2), LTA_4 synthetase; (3), LTA_4 hydrolase; (4), LTB_4 ω-hydroxylase (P-450 LTB_ω); (5), dehydrogenase(?); HPETE, hydroperoxyeicosatetraenoic acid; LTA_4, leukotriene A_4.

2. Biological significance of ω-type oxidation

As mentioned earlier, the biological significance of ω-oxidation of fatty acids have been elucidated in animals, including humans and insects as well as plants. Interestingly, the biological significance of (ω-1)-oxidation of fatty acids was confirmed in insects. Although other ω-type oxidations of fatty acids, that is, (ω-2), (ω-3) and (ω-4) oxidation of fatty acids, were found in microorganisms[24)–26)] and plants,[27)] their biological significance has not been yet to be determined. The author speculates that ω-type oxidation of fatty acids might be a reaction step to produce biologically active fatty acid derivatives. However, such derivatives have not been yet to be found. The author is hopeful that these derivatives will be discovered in the near future. Further experimental work is needed to more clearly elucidate the biological significance of ω-type oxidation of fatty acids and their derivatives such as PG, LT, prostacyclin and thromboxane.

3. Conclusion

Since comprehensive studies on the biological significance of ω-oxidation of fatty acids are not available, the author examined the significance of this process from a wide variety of viewpoints. ω-Oxidation of fatty acids is an alternative oxidation pathway for β-oxidation when β-oxidation is blocked. The increase in ω-oxidation of fatty acids under conditions of starvation or diabetes may contribute to the production of glucose from succinyl-CoA. Also fatty acid ω- and (ω-1)-oxidation is an essential reaction for the production of insect pheromones in honeybees and the formation of biopolyesters in higher plants. The author observes that the total hydroxylation activity (*i.e.*, the sum of ω- and (ω-1)-hydroxylation activity of lauric acid or tridecanoic acid) is correlated to energy production in non-traditional laboratory animals such as the musk shrew, Mongolian gerbil, harvest mouse and triton hamster. The ω-hydroxylation of very long chain fatty acids is an important reaction in the formation of hydroxyceramides in the epidermis. Finally, it is inferred that ω-oxidation of PGs and LTs contributes to the regulation of the biological activities of these (e)icosanoids.

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References

1) Fulco, A.J. (1967) 2-Hydroxylation and decarboxylation of long chain fatty acids by yeast. J. Biol. Chem. **242**, 3608–3613.
2) Hitchcock, C., Morris, L.J. and James, A.T. (1968) The stereochemistry of α-oxidation of fatty acids in plants. Eur. J. Biochem. **3**, 419–421.
3) Hoshi, M. and Kishimoto, Y. (1973) Synthesis of cereboronic acid from lignoceric acid by rat brain preparation: Some properties and distribution of α-hydroxylation system. J. Biol. Chem. **248**, 4123–4130.
4) Herndon, J.H. Jr., Steinberg, D., Uhlemdorf, B.W. and Fales, H.M. (1969) Refsum's disease: Characterization of the enzyme defect in cell culture. J. Clin. Invest. **48**, 1017–1032.
5) Verkade, P.E., Elzas, M., van der Lee, J., DeWolff, H.H., Verkade-Sandbergen, A. and van der Sande, D. (1933) Untersuchungen uber der Fett Stoffwechsel. Z. Phys. Chem. **215**, 225–257.
6) Verkade, P.E. and van der Lee, J. (1934) Researches on fat metabolism. II. Biochem. J. **28**, 31–39.
7) Verkade, P.E. (1938) The role of the dicarboxylic acid in metabolism. Chem. & Ind. (London) **57**, 704–711.
8) Wakabayashi, K. and Shimazono, N. (1961) Studies *in vitro* on the mechanism of ω-oxidation of fatty acids. Biochim. Biophys. Acta **48**, 615–617.
9) Robbins, K.C. (1968) *In vitro* ω-oxidation of medium chain fatty acids on mammalian tissue. Arch. Biochem. Biophys. **123**, 531–538.
10) Lu, A.Y.H. and Coon, M.J. (1968) Role of hemoprotein P-450 in fatty acid ω-hydroxylation in a soluble enzyme system from liver microsomes. J. Biol. Chem. **243**, 1331–1332.
11) Wada, F., Shibata, H., Goto, M. and Sakamoto, Y. (1968) Participation of the microsomal electron transport system involving cytochrome P-450 in ω-oxidation of fatty acids. Biochim. Biophys. Acta **162**, 518–524.
12) http://www.cypalleles.ki.se/.
13) Reuttinger, R.T., Olson, S.T., Boyer, R.F. and Coon, M.J. (1974) Identification of the ω-hydroxylase of *Pseudomonas oleovorans* as a nonheme iron protein requiring phospholipid for catalytic activity. Biochem. Biophys. Res. Commun. **57**, 1011–1017.
14) Ichihara, K., Kusunose, E. and Kusunose, M. (1969) Some properties and distribution of the ω-hydroxylation system of medium-chain fatty acids. Biochim. Biophys. Acta **176**, 704–712.
15) Miura, Y., Hisaki, H. and Ueta, N. (1978) ω- and (ω-1)-hydroxylation of fatty acids by frog liver microsomes. Biochim. Biophys. Acta **531**, 149–157.
16) Miura, Y., Hisaki, H. and Ueta, N. (1980) Effect of detergents on the microsomal cytochrome P-450-dependent monooxygenases from frog and rat liver. Comp. Biochem. Physiol. **67B**, 541–547.
17) Miura, Y. (1981) ω- and (ω-1)-hydroxylation of 1-dodecanol by frog liver microsomes. Lipids **16**, 721–725.
18) Miura, Y. (1982) Effect of inhibitors on ω- and (ω-1)-hydroxylation of lauric acid by frog liver microsomes. Lipids **17**, 864–869.
19) Miura, Y. (1982) ω- and (ω-1)-hydroxylation of lauric acid by frog liver microsomes. *In* Microsomes, Drug Oxidations and Drug Toxicity (eds. Sato, R. and Kato, R.). Japan Scientific Societies Press, Tokyo, pp. 301–302.
20) Miura, Y. (1985) Seasonal changes and sex difference in laurate hydroxylase activity in frog liver microsomes. Comp. Biochem. Physiol. **82C**, 63–67.
21) Bertrand, J.C., Bazin, H., Zacek, M., Gilewicz, M. and Azoulay, E. (1979) NADPH-cytochrome *c* reductase of *Candida tropicalis* grown on alkane. Eur. J. Biochem. **93**, 237–243.
22) Benveniste, I., Salaun, J.P. and Durst, F. (1977) Wounding-induced cinnamic acid hydroxylase in

Jerusalem artichoke tuber. Phytochemistry **16**, 69–73.
23) Capdevila, J., Ahmad, N. and Agosin, M. (1975) Soluble cytochrome P-450 from housefly microsomes: Partial purification and characterization of two hemoprotein forms. J. Biol. Chem. **250**, 1048–1060.
24) Miura, Y. and Fulco, A.J. (1974) (ω-2) Hydroxylation of fatty acids by a soluble system from *Bacillus megaterium*. J. Biol. Chem. **249**, 1880–1888.
25) Miura, Y. and Fulco, A.J. (1975) (ω-1), (ω-2) and (ω-3) hydroxylation of long chain fatty acids, amides and alcohols by a soluble enzyme system from *Bacillus megaterium*. Biochim. Biophys. Acta **388**, 305–317.
26) Shoun, H., Sudo, Y. and Beppu, T. (1985) Subterminal hydroxylation of fatty acids by a cytochrome P-450-dependent enzyme system from a fungus, *Fusarium oxysporm*. J. Biochem. **97**, 755–763.
27) Salaun, J.-P., Benveniste, I., Reichhart, D. and Durst, F. (1978) A microsomal (cytochrome P-450)-linked lauric acid monooxygenase from aged Jerusalem Artichoke-tuber tissues. Eur. J. Biochem. **90**, 155–159.
28) Wen, L.-P. and Fulco, A.J. (1987) Cloning of the gene encoding a catalytically self-sufficient cytochrome P-450 fatty acid monooxygenase induced by barbiturates in *Bacillus megaterium* and its functional expression and regulation in heterogus (*E. coli*) and homologus (*B. megaterium*) hosts. J. Biol. Chem. **262**, 6676–6682.
29) Yamakawa, T. (1950) The fate of branched chain fatty acids in animal body IV. A contribution of the mode of biological breakdown of naturally occurring fatty acid. J. Biochem. **37**, 343–353.
30) Weitzel, G. (1951) Biochimieverzweigter Carbonsauren VI. Mitteilung Stoffwechselversuche mit alkalverzweigten hoheren Fettsauren. Hoppe-Seyl. Z. Phys. Chem. **287**, 254–295.
31) Bergstrom, S., Borgstrom, B., Tryding, N. and Westoo, G. (1954) Intestinal absorption and metabolism of 2:2-demethylstearic acid in rat. Biochem. J. **58**, 604–608.
32) Flaschentrager, B. and Bernhard, K. (1935) Uber den biologischen Abbau von Fettsauren, Estern und Fett zu Dicarbonsauren. Helv. Chim. Acta **18**, 962–972.
33) Bjorkhem, I. (1973) ω-Oxidation of stearic acid in the normal, starved and diabetic rat liver. Eur. J. Biochem. **40**, 415–422.
34) Bjorkhem, I. (1976) On the mechanism of regulation of ω-oxidation of fatty acids. J. Biol. Chem. **251**, 5259–5266.
35) Bjorkhem, I. (1978) On the quantitative importance of ω-oxidation of fatty acids. J. Lipid Res. **19**, 585–590.
36) Wada, F. and Usami, M. (1977) Studies on fatty acid ω oxidation. Biochim. Biophys. Acta **487**, 261–268.
37) Gregersen, N. and Ingerslev, J. (1979) The excretion of C_6-C_{10}-dicarboxylic acids in the urine of newborn infants during starvation: Evidence for ω-oxidation of fatty acids in the newborn. Acta Paediatr. Scand. **68**, 677–681.
38) Mortensen, P.B. (1980) The possible antiketogenic and gluconeogenic effect of the ω-oxidation of fatty acids in rats. Biochim. Biophys. Acta **620**, 177–185.
39) Kam, W., Kumaran, K. and Landau, B.R. (1978) Contribution of ω-oxidation to fatty acid oxidation by liver of rat and monkey. J. Lipid Res. **19**, 591–600.
40) Hemmelgarn, E., Schumann, W.C., Margolis, J., Kumaran, K. and Landau, B.R. (1979) ω-Oxidation of fatty acids and the acetylation of *p*-aminobenzoic acid. Biochim. Biophys. Acta **572**, 298–306.
41) Petter, F. (1967) The care and management of laboratory animals. *In* The UFAW Handbook. 3rd ed., E. & S. Livingstone Ltd., Edinburgh and London, pp. 449–451.
42) Oda, S. and Kondo, K. (1977) Novel laboratory animal of the order Insectivora. Exp. Animal (Tokyo) **26**, 273–280.
43) Kondo, K. (1985) The aspects and reproduction of novel laboratory animals. *In* Novel Laboratory Animals (eds. Oda, S., Kito, J. and Isomura, G.). Japan Scientific Societies Press, Tokyo, pp. 1–7.
44) Oda, S., Okada, Y.S. and Kameyama, Y. (1982) Teratogenic susceptibility of the house musk shrew, *Suncus murinus*, to vitamin A. Environ. Med. **26**, 52–57.
45) Nagai, K., Ishizuka, I. and Oda, S. (1984) Acidic glycolipids from kidney of suncus (Insectivora). J. Biochem. **95**, 1501–1511.
46) Otsuru, O., Otsuka, H., Kasama, T., Seyama, Y., Sakai, T. and Yohro, T. (1983) The characterization of 2,3-alkanediol diacylesters obtained from the harderian glands of Mongolian gerbil (*Meriones unguiculatus*). J. Biochem. **94**, 2049–2054.
47) Leach, A.B. and Holub, B.J. (1984) The effect of dietary lipid on the lipoprotein status of the Mongolian gerbil. Lipids **19**, 25–33.
48) Walker, E.P. (1983) Common, black-bellied, or ratlike hamsters *In* Mammals of the World. 4th ed. (eds. Nowak, R.M. and Praradiso, J.L.). The Johns Hopkins University Press, Baltimore and London, pp. 622–623.
49) Miura, Y., Hisaki, H. and Oda, S. (1984) Cytochrome P-450-dependent fatty acid hydroxylase system in Japanese house musk shrew, *Suncus murinus*. J. Biochem. **95**, 779–784.
50) Miura, Y. and Oda, S. (1994) Oxygenation of unsaturated fatty acids by hepatic microsomes of musk shrew (*Suncus murinus*). Comp. Biochem. Physiol. **108B**, 21–25.
51) Miura, Y., Hisaki, H., Siems, W. and Oda, S. (1987) Hydroxylation of fatty acids and alcohols by hepatic microsomal cytochrome P-450 system from the Mongolian gerbil. Lipids **22**, 987–993.
52) Miura, Y., Hisaki, H. and Oda, S. (1990) Fatty acid

hydroxylase system in the Japanese harvest mouse, *Micromys minutus*. Lipids **25**, 78–81.
53) Miura, Y. and Oda, S. (2000) Fatty acids hydroxylase system in triton hamster, *Cricetulus triton*. Abstracts of Japan Oil Chemists' Society and American Oil Chemists' Society World Congress 2000 (Kyoto, Japan), p. 83.
54) Miura, Y., Hisaki, H., Fukushima, B., Oda, S. and Nagai, T. (1991) Comparative aspects of microsomal cytochrome P-450-dependent monooxygenase activities in musk shrew (*Suncus murinus*), Mongolian gerbil (*Meriones unguiculatus*), harvest mouse (*Micromys minutus*) and rat. Comp. Biochem. Physiol. **100B**, 249–252.
55) Miura, Y., Oda, S. and Suzuki, H. (2007) Physiological significance of ω- and (ω-1)-hydroxylation of fatty acids in novel laboratory animals. Abstracts of 98th American Oil Chemists' Society Annual Meeting (Quebec City, Canada), p. 37.
56) Adolph, E.F. (1949) Quantitative relations in the physiological constitutions of mammals. Science **109**, 579–585.
57) Stahl, W.R. (1962) Similarity and dimensional methods in biology. Science **137**, 205–212.
58) Calder, W.A. III (1984) Physical and quantitative background. *In* Size, Function and Life History. Harvard Univ. Press, Cambridge and London, pp. 25–33.
59) Hamanaka, S., Suzuki, M., Suzuki, A. and Yamakawa, T. (2001) Epidermosides: Structure and function of skin-specific glycolipids. Proc. Jpn. Acad., Ser. B. **77**, 51–56.
60) Gray, G.M., White, R.J. and Majer, J.R. (1978) 1-(3′-*O*-acyl)-*β*-glucosyl-*N*-dihydroxypentatriacontadienoylsphingosine, a major component of the glucosylceramides of pig and human epidermis. Biochim. Biophys. Acta **528**, 127–137.
61) Bowser, P.A., Nugteren, D.H., White, R.J., Houtsmuller, U.M.T. and Prottey, C. (1985) Identification, isolation and characterization of epidermal lipids containing linoleic acid. Biochim. Biophys. Acta **834**, 419–428.
62) Hamanaka, S., Asagami, C., Suzuki, N., Inagaki, F. and Suzuki, A. (1989) Structure determination of glucosyl *β* 1-*N*-(ω-*O*-linoleoyl)-acylsphingosines of human epidermis. J. Biochem. **105**, 684–690.
63) Behne, M., Uchida, Y., Seki, T., Ortiz de Montellano, P., Elias, P.E. and Holleran, W.M. (2000) Omega-hydroxyceramides are required for corneocyte lipid envelop (CLE) formation and normal epidermal permeability barrier function. J. Invest. Dermatol. **114**, 185–192.
64) Lercker, G., Capella, P., Conte, L.S. and Ruini, F. (1981) Components of royal jelly: I. Identification of the organic acids. Lipids **16**, 912–918.
65) Plettner, E., Slessor, K.N., Winston, M.L. and Oliver, J.E. (1996) Casteselective pheromone biosynthesis in honeybees. Science **271**, 1851–1853.
66) Robinson, G.E. (1996) Chemical communication in honeybees. Science **271**, 1824–1825.
67) Kamakura, M. (2011) Royalactin induces queen differentiation in honeybees. Nature **473**, 478–483.
68) Kolattukudy, P.E. (1980) Biopolyester membranes of plants: Cutin and suberin. Science **208**, 990–1000.
69) Kolattukudy, P.E. and Walton, T.J. (1972) Structure and biosynthesis of the hydroxyl fatty acids of cutin in *Vicia faba* leaves. Biochemistry **11**, 1897–1907.
70) Kupfer, D. (1980) Endogenous substrate of monooxygenases: Fatty acids and prostaglandins. Pharmacol. Ther. **11**, 469–496.
71) Powell, W.S. (1978) ω-Oxidation of prostaglandins by lung and liver microsomes. J. Biol. Chem. **253**, 6711–6716.
72) Yamamoto, S., Kusunose, E., Ogita, K., Kaku, M., Ichihara, K. and Kusunose, M. (1984) Isolation of cytochrome P-450 highly active in prostaglandin ω-hydroxylation from lung microsomes of rabbits treated with progesterone. J. Biochem. **96**, 593–603.
73) Yamamoto, S., Kikuta, Y. and Kusunose, M. (1995) Metabolism of unsaturated fatty acid by cytochrome P-450. *In* AA, EPA, DHA-Highly Unsaturated Fatty Acids (ed. Kayama, M.). Koseisha-Koseikaku, Tokyo, pp. 96–115.
74) Hansson, G., Lindgren, J.A., Dahlen, S.E., Hedqvist, P. and Samuelsson, B. (1981) Identification and biological activity of novel ω-oxidized metabolites of leukotriene B_4 from human leukocytes. FEBS Lett. **130**, 107–112.
75) Shak, K. and Goldstein, I.M. (1985) Leukotriene B_4 ω-hydroxylase in human polymorphonuclear leukocytes. J. Clin. Invest. **76**, 1218–1228.
76) Sumimoto, H., Takeshige, K. and Minakami, S. (1988) Characterization of human neutrophil leukotriene B_4 ω-hydroxylase as a system involving a unique cytochrome P-450 and NADPH-cytochrome P-450 reductase. Eur. J. Biochem. **172**, 135–324.

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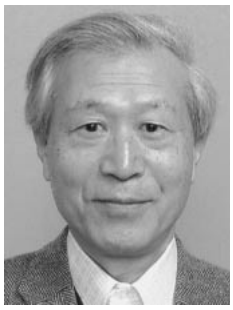
Profile



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