# Effects of caffeoyl- and galloyl-tannins on arachidonate metabolism in rat peritoneal macrophage homogenates

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## Abstract

Studies were made on the effects of three caffeoyltannins such as caffeolymalic acid, caffeoyltartaric acid and rosmarinic acid, and two galloyltannins such as 3-*O*-digalloylquinic acid and 2.6-digalloyl-1,5-anhydro-D-glucitol (acertannin), on the formation of the 5-lipoxygenase product, 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) and the cyclooxygenase products, 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), thromboxane $B_2$ and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in a homogenate of rat peritoneal macrophages. 3-*O*-Digalloylquinic acid, acertannin, caffeoylmalic acid, caffeoyltartaric acid and rosmarinic acid selectively inhibited the formations of the 5-lipoxygenase product 5-HETE in the homogenate. Their concentrations for 50 % inhibition ($IC_{50}$) of 5-HETE formation were 7.00, 22.5, 425.0, 42.0 and 6.90 $\mu$M, respectively. At concentrations of $10^{-4}$M –$10^{-5}$M, the five tannins tested in this study, increased the formation of the cyclooxygenase products HHT, thromboxane $B_2$ and 6-keto-$PGF_{1\alpha}$.At concentrations of $10^{-3}$M, 3-*O*-digalloylquinic acid inhibited the formations of HHT and thromboxane $B_2$, but it increased the formation of 6-keto-$PGF_{1\alpha}$.

**Key words** caffeoyl-tannin, galloyl-tannin, 5-lipoxygenase, arachidonate metabolism, rat macrophage.

**Abbreviations** 5-HETE, 5-hydroxy-6,8,11,14-eicosatetraenoic acid; HHT, 12-hydroxy-5,8,1-heptadecatrienoic acid; $TXB_2$, thromboxane $B_2$; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$.

## Introduction

Leukotrienes participate in immunoregulation and in a variety of diseases, including asthma, inflammation and various allergic conditions. In the presence of 5-lipoxygenase, free arachidonic acid is converted to 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE), which is then reduced to 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) or dehydrated to an unstable intermediate, leukotriene $A_4$ ($LTA_4$).[1,2] $LTA_4$ can be further converted enzymatically to leukotriene $B_4$ ($LTB_4$) and leukotrine $C_4$ ($LTC_4$).[3,4] The slow reacting substance of anaphylaxis (SRS-A) is formed from arachidonic acid in the presence of 5-lipoxygenase.[5] Furthermore, 5-HPETE and 5-HETE enhance histamine release induced by antigen from human basophilic leukocytes.[6] 5-HETE increased the release of lysosomal enzymes such as $\beta$-glucuronidase and lysozyme induced by platelet activating factor (PAF) in human polymorphonuclear leukocytes.[7] Therefore, specific inhibitors of 5-lipoxygenase should be useful not only in the therapy of allergic diseases such as asthma and of inflammation, but also in studies on the biosynthesis and functions of leukotrienes.

On the other hand, cyclooxygenase in rat peritoneal macrophage is known to catalyze the initial reaction that leads to the formation of prostaglandin $H_2$ ($PGH_2$), which is converted to thromboxane $A_2$

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($TXA_2$) by thromboxane synthetase and to other eicosanoids such as prostaglandin $D_2$ ($PGD_2$) and prostaglandin $E_2$ ($PGE_2$).[8] $TXA_2$, which causes platelet aggregation,[9] is readily converted to thromboxane $B_2$ ($TXB_2$), which is stable. In this sutdy, we confirmed the formation of 6-keto-prostaglandin F1$\alpha$ (6-keto-PGF1$\alpha$) from arachidonic acid in homogenates of rat peritoneal macrophages. Prostaglandin $I_2$ ($PGI_2$), which is formed from $PGH_2$ by prostacyclin synthetase,[10] is vasodilator and inhibits platelet activation.[11] $PGI_2$ changes spontaneously 6-keto-PGF1$\alpha$, which is stable.

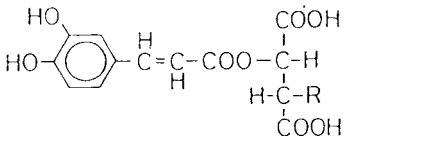
As a part of series of biological examinations of various tannins and related compounds,[12-15] we already reported that tannins and related compounds inhibit the lipid peroxidation in rat liver, and prevent the liver injury and hyperlipemia induced by oral administration of peroxidized oil. Medicinal plants containing tannins and related compounds, which are used in this study, have been traditionally used in treatment of allergic and inflammatory diseases in Japan and China. In the present work, we investigated the inhibitory effects of five tannins on the formation of 5-HETE (5-lipoxygenase product) and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), $TXB_2$ and 6-keto-PGF1$\alpha$ (cyclooxygenase products) from arachidonic acid in rat peritoneal macrophages.

## Materials and Methods

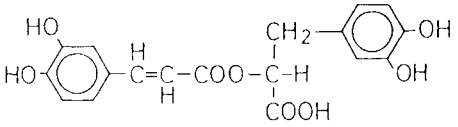
*Materials* : [1-$^{14}$C] Arachidonic acid (specific activity : 59.6 mCi / mol = 2016.5 MBq / mmol) was obtained from the Radiochemical Centre, Amersham (Japan). [$^3$H] 6-Keto-PGF1$\alpha$ and [$^3$H] $TXB_2$ were purchased from New England Nuclear (Japan). 5-HETE and $LTB_4$ were purchased from Funakoshi Co. (Japan). Precoated silica gel 60 TLC plastic sheets were obtained from Merck Co. (Japan). Eagle's minimum essential medium (EMEM) and fetal bovine serum (FBS) were purchased from Nissui Pharmaceutical Co. Ltd. (Japan). Other chemicals were reagent grade.

Caffeoylmalic acid was isolated from the leaves of *Acalpha australis* L. by Okuda *et al.*.[16] Caffeoyltartaric acid and rosmarinic acid were isolated from the leaves of *Rabdosia japonica* HARA and *Perilla frutescens* BRITTON var. *crispa* DECNE, respectively, by Okuda *et al.*.[17] 3-*O*-Digalloylquinic acid and acertannin were isolated from the leaves of *Koelreuteria paniculata* LAXIM. and *Acer ginala* MAXIM., respectively, by Okuda *et al.*.[18,19] The structures of these compounds are shown in Fig. 1. Test compounds were dissolved in Hepes/saline buffer (25 mM Hepes in 135 mM NaCl, pH 7.4) before use.

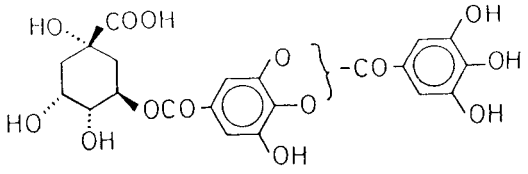
*Preparation of rat peritoneal macrophages* : Rat peritoneal cells were prepared by the modification of the method of Borgeat and Samuelsson.[20] Wistar-King strain rats (250-400 g) were sacrificed by decapitation 3-4 hr after intraperitoneal treatment with 0.2 % glycogen / 0.9 % NaCl solution (40 ml / rat)



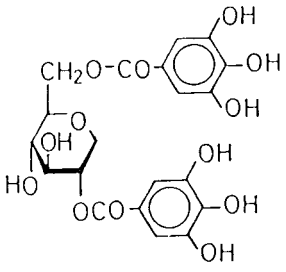

Caffeoylmalic Acid : R=H
Caffeoyltartaric Acid : R=OH




Rosmarinic Acid




3-Digalloylquinic Acid




Acertannin

Fig. 1 Chemical structures of test compounds.

containing heparin (5 I.U/ml). Peritoneal cells were collected from the peritoneal cavity, centrifuged at 400×g and 4°C for 15 min, and treated with 0.3 % NaCl solution to lyze contaminating red cells. Then, peritoneal cells were suspended in EMEM containing 5 % FBS in $3\times10^6$ cells/ml EMEM. The cells were seeded on the culture dish (35 mm$\phi$) and were incubated in 5 % $CO_2$/95 % air at 37°C for 2 hrs. Then the dish was washed vigorously with $Ca^{2+}$ free Hepes/saline buffer to remove non-adherent cells and suspended in Hepes/saline buffer.

*Measurement of [1-$^{14}$C] arachidonic acid metabolites in homogenates of rat peritoneal macrophages* : Preparations of rat peritoneal macrophages were sonicated in a Sonifier Cell Disruptor (Bransor Sonic Power Co.), and the sonicated preparations (10 mg protein/ml) were preincubated with test compounds and 2.0 mM $CaCl_2$ for 5 min at 37°C. Then, [1-$^{14}$C] arachidonic acid (0.1 $\mu$Ci=3.7 KBq) was added at a final concentration of 1.67 nmoles (153250 cpm/0.4 ml/tube) and the mixture was incubated for 5 min at 37°C. The reaction was stopped by adding 0.5 N formic acid and the mixture was extracted with 8 volumes of ethylacetate. The ethylacetae phase was evaporated under a nitrogen stream, and the residue was dissolved in a small amount of ethylacetate (40 $\mu$l), applied to precoated silica gel thin layer chromatography (TLC) plastic sheets, and developed with two solvents : ethylacetate-2,2,4-trimethylpentane-acetic acid-distilled water (100 : 50 : 20 : 100, v/v, upper phase) and chloroform-methanol-acetic acid-distilled water (130 : 120 : 15 : 12, v/v). Metabolites were identified by comparison of their mobilities with those of authentic samples and by gas-mass (GC-MS) spectrometry as described previously.[21,22] Spots of radioactivity were detected by autoradiography, cut out with scissors and counted in a liquid scintillation counter. Protein was determined by the method of Lowry *et al.*[23] with bovine serum albumin as a standard.

*Measurement of free radical scavenging effects* : The indicated amounts of test compounds were incubated with $10^{-4}$M 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in ethanol for 5 min at room temperature, and then the optical density at 520 nm (O.D. 520) of the reaction mixture was measured. The amounts of DPPH radical in the presence and absence of test compounds were determined from the decrease of O. D. 520 and expressed as percentage of the control value.

## Results

*Eeffects of tannins and related compounds on arachidonate metabolism in rat peritoneal macrophage homogenates*

When arachidonic acid was incubated with a homogenate of rat peritoneal macrophages, it was converted to the cyclooxygenase products HHT, $TXB_2$ and 6-keto-$PGF1\alpha$ and the 5-lipoxygenase product 5-HETE. The radioactivities of HHT, $TXB_2$, 6-keto $PGF1\alpha$ and 5-HETE in control mixtures were 17.0±0.79, 14.9±0.64, 10.6±0.75 and 6.86±0.20 ($\times10^3$ cpm) (means±standard errors for 15 experiments), respectively.

Fig. 2 shows the dose-dependence of the effects of 3-*O*-digalloylquinic acid, acertannin, rosmarinic acid, caffeoyltartaric acid and caffeoylmalic acid on the formations of the lipoxygenase product, 5-HETE, and the cyclooxygenase products HHT, $TXB_2$ and 6-keto-$PGF1\alpha$.

3-*O*-Digalloylquinic acid, at concentrations of $10^{-6}$M-$10^{-4}$M, inhibited the formation of 5-HETE, but it stimulated the formations of HHT, $TXB_2$ and 6-keto-$PGF1\alpha$, at concentrations of $10^{-5}$M-$10^{-4}$M. Moreover, at a concentration of $10^{-3}$M, 3-*O*-digalloylquinic acid inhibited the formtaion of 5-HETE, HHT and $TXB_2$, but it stimulated the formation of 6-keto-$PGF1\alpha$ (Fig. 2a).

As shown in Fig. 2b and 2c, acertannin and rosmarinic acid also inhibited the formation of 5-HETE, dose-dependently. At concentrations of $10^{-4}$M, they stimulated the formations of HHT, $TXB_2$ and 6-keto-$PGF1\alpha$, but they strongly inhibited the formations of both cyclooxygenase and 5-lipoxygenase products at concentrations of $10^{-3}$M (Fig. 2b and 2c).

Caffeoyltartaric acid also inhibited the formation of 5-HETE, dose-dependently, but it stimulated the formations of the cyclooxygenase products HHT, $TXB_2$ and 6-keto-$PGF1\alpha$ at concentrations of $10^{-4}$M-$10^{-3}$M. At a concentration of $10^{-3}$M, its stimulatory effect on the formation of 6 keto-$PGF1\alpha$ was stron-

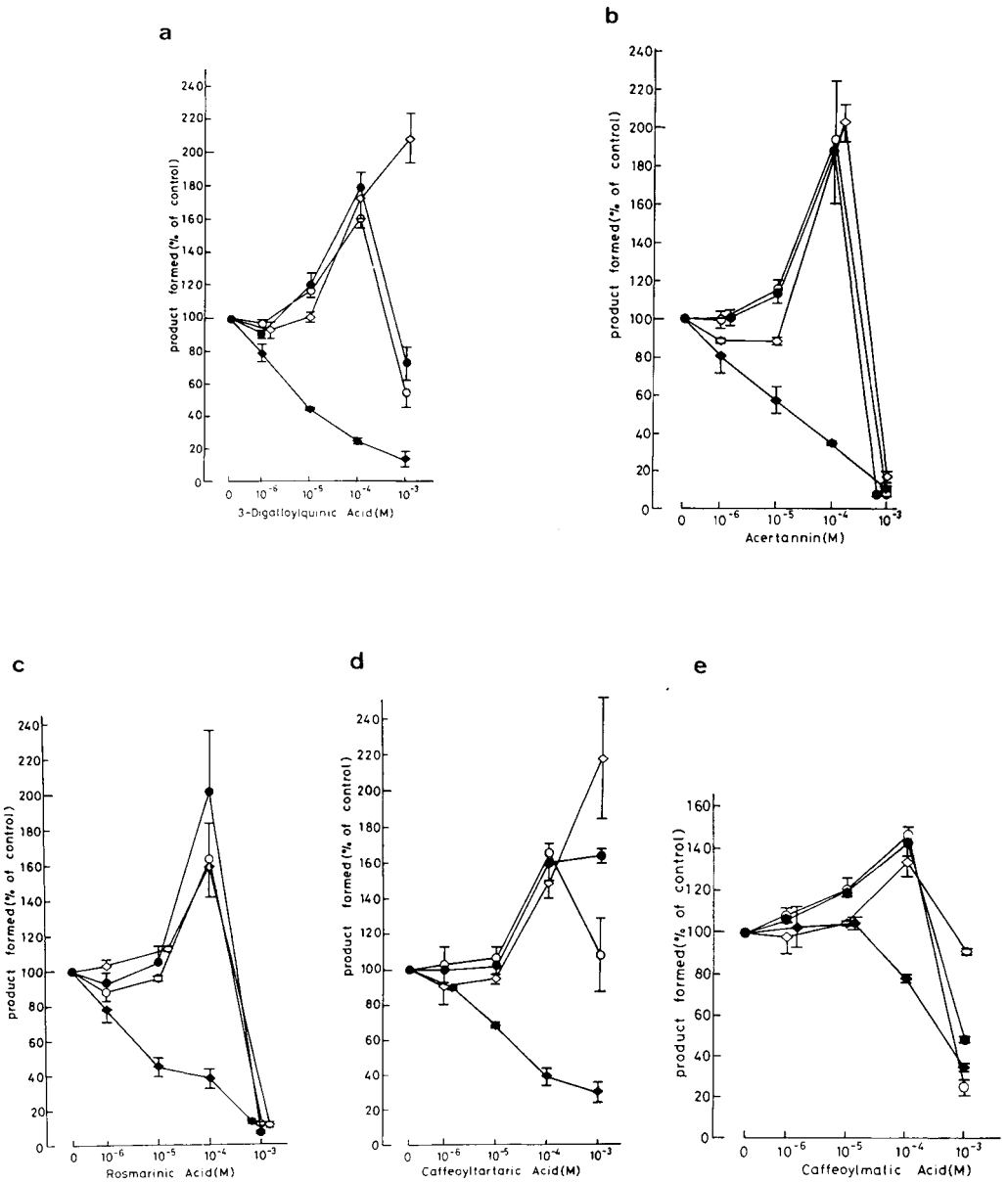



Fig. 2 Effects of 3-*O*-digalloylquinic acid (Fig. 2a), acertannin (Fig. 2b), rosmarinic acid (Fig. 2c), caffeoyltartaric acid (Fig. 2d) and caffeoylmalic acid (Fig. 2e) on the formations of 5-HETE, HHT, thromboxane $B_2$ and 6-keto-prostagalndin $F1\alpha$ from free arachidonic acid in homogenates of rat peritoneal macrophages.
Values are means±S.E. for 3 experiments.
●, thromboxane $B_2$ ; ○, HHT ; ◇, 6-keto-prostaglandin $F1\alpha$ ; ◆, 5-HETE

ger than those on the formations of HHT and $TXB_2$ (Fig. 2d).

Caffeoylmalic acid also inhibited the formation of 5-HETE at concentrations of $10^{-4}$M - $10^{-3}$M, but it stimulated the formations of HHT and $TXB_2$, dose-dependently, at concentrations of $10^{-6}$M - $10^{-4}$M. At the concentration of $10^{-3}$M, caffeoylmalic acid inhibited the formations of 5-HETE, HHT and $TXB_2$ without affecting 6-keto-$PGF1\alpha$ formation (Fig. 2e). Therefore, the $IC_{50}$ values of 3-*O*-digalloylquinic acid, acertannin, rosmarinic acid, caffeoyltartaric acid and caffeoylmalic acid for the formation of 5-HETE were

7.00, 22.5, 6.90, 42.0 and 425 $\mu$M, respectively.

*Free radical scavenging effect of tannins and related compounds*

As shown in Fig. 3, all these compounds had strong radical scavenging actions. The free radical scavenging action of these tannins was in the order acertannin > 3-*O*-digalloylquinic acid > rosmarinic acid > caffeoyltartaric acid > caffeoylmalic acid.

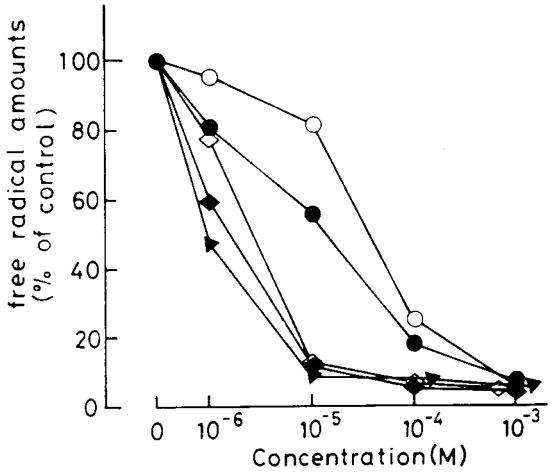



Fig. 3 Effects of 3 *O* digalloylquinic acid, acertannin, rosmarinic acid, caffeoyltartaric acid and caffeoylmalic acid on 1,1 diphenyl-2-picrylhydrazyl radical (DPPH radical)
◆, 3 *O* digalloylquinic acid; ▲, acertannin; ◇, rosmarinic acid; ●, caffeoyltartaric acid; ○, caffeoylmalic acid

## Discussion

In this study, we detected the formations of cyclooxygenase products (HHT, $TXB_2$ and 6-keto-$PGF_{1\alpha}$) and 5-lipoxygenase product (5-HETE) in a rat peritoneal macrophage homogenate. It is generally known that tannins are strongly bound to enzymes, and that they act as the non-specific inhibition for enzyme activity, consequently. In this study, 3-*O*-digalloylquinic acid, acertannin, rosmarinic acid and caffeoylmalic acid inhibited both 5-lipoxygenase and cyclooxygenase at the high concentration of $10^{-3}$M. Therefore, it is suggested that these actions by the above tannins may be due to non-specific inhibition for enzymes. However, Kakiuchi *et al.*[24] reported that the inhibition for glucosyltransfease by galloyl tannins was not caused by the non-specific binding of tannins and proteins. Furthermore, we found that hydrolyzable tannins stimulated adrenocorticotropic hormone (ACTH)-induced lipolysis in fat cells at concentrations of 5-20 $\mu$g/ml, while they inhibited adrenaline induced lipolysis at the above same concentrations.[25,26] Thus, it seems unlikely that various tannins are non specific inhibitors for enzymes at low concentrations. The present experiments showed that 3-*O*-digalloylquinic acid, acertannin, rosmarinic acid, caffeoyltartaric acid and caffeoylmalic acid selectively inhibited the formations of 5-lipoxygenase product, 5-HETE at concentrations of $10^{-5}$M-$10^{-4}$M, while these tannins stimulated the formation of cyclooxygenase products HHT, $TXB_2$ and 6-keto-$PGF_{1\alpha}$ at the above same concentrations. These results suggest that the inhibition of 5-lipoxygenase by these tannins, at concentrations of $10^{-5}$M-$10^{-4}$M, might be due to specific inhibition for 5-lipoxygenase rather than non-specific inhibition. Consequently, the stimulation by these tannins of HHT, $TXB_2$ and 6-keto-$PGF_{1\alpha}$ formations may be due to an increase in the amount of substrate available for the cyclooxygenase system by their inhibition of the 5-lipoxygenase system.

In addition, the tannins and related compounds examined in this study were all found to have potent free radical scavenging actions. The strength for inhibitory effects of these tannins and related compounds on 5-lipoxygenase was similar to those for free radical scavenging effects. These findings suggest that 5-lipoxygenase inhibitions by these tannins are closely associated with free radical scavenging actions by them. Okuda *et al.* reported that various tannins isolated from medicinal plants form themselves into stable radicals by the methods using electron spin resonance (ESR), and suggesting that the stable radical forms of these tannins scavenge the radicals such as ·OH and $O_2^-$ generated by biological oxidation, consequently.[27,28] On the other hand, it has been reported that $PGE_2$ synthesis *via* cyclooxygenase was stimulated by some radical scavengers, probably by preventing inactivation of the enzyme by oxygen radicals.[29,30] Therefore, another mechanism for the stimulatory effects of these tannins on the formations of cyclooxygenase products HHT, $TXB_2$ and 6 keto-$PGF_{1\alpha}$ could be explained by potent radical scavenging activity of these tannins.

From these results, it is suggested that 5-lipoxygenase inhibition and cyclooxygenase stimulation by these tannins are closely associated with free radical scavenging actions by them.

It has been reported that norepinephrine stimulates the formations of cyclooxygenase products $PGE_2$ and 6-keto-$PGF_{1\alpha}$ in rat stomach[31,33] and rat adipocytes.[34] From the structure-relationship between these tannins and norepinephrine, it seems likely that the adjacent phenolic hydroxyl groups of these test compounds are identical with those of norepinephrine, and essential for stimulation of cyclooxygenase activity. Previously, we reported that baicalein (5,6,7-trihydroxyflavone), esculetin (6,7-dihydroxycoumarin) and daphnetin (7,8-dihydroxycoumarin) having adjacent phenolic hydroxyl groups in the skeletons selectively inhibited 5-lipoxygenase.[22,35] Thus, it is suggested that adjacent phenolic hydroxyl group must be essential for potent inhibition of 5-lipoxygenase activity. The relationship between the 5-lipoxygenase inhibition, the cyclooxygenase stimulation and the radical scavenging action by various tannins remains to be clarified by further work.

A number of non-steroidal anti-inflammatory drugs such as aspirin and indomethacin have been used as antipyretic, antiphlogistic and analgesic, which have been shown to inhibit the cyclooxygenase but not lipoxygenase.[9,36] Medicinal plants containing tannins and related compounds, which are used in this study, have been traditionally used for treatment of allergic inflammation such as asthma, chronic hepatitis, diarrhea and inflammatory bowel diseases in Japan and China. Leukotrienes (5-lipoxygenase products) formed from free arachidonic acid in polymorphonuclear neutrophils, basophils and eosinophils are related to allergic diseases, especially, asthma, atopic dermatitis and chronic hepatitis. $LTB_4$ (a 5-lipoxygenase product) is known to be potent chemotactic substances[37] and to cause leukocyte degranulation.[38,39] Recently, it has been reported that $LTB_4$ is related to inflammatory bowel diseases.[40] Therefore, it seems likely that medicinal plants containing 3-*O*-digalloylquinic acid, acertannin, rosmarinic acid, caffeoyltartaric acid and caffeoylmalic acid may be useful as therapeutic drugs for treatment of allergic inflammation, especially, asthma, atopic dermatitis and inflammatory bowel diseases rather than those for the antipyretic, antiphlogistic and analgesic actions. Further research is needed to clarify the significances of these compounds *in vivo*.

## 和文抄録

3種のカフェー酸誘導体 caffeoylmalic acid, caffeoyltartaric acid, rosmarinic acid, 2種の galloyl 誘導体 3-*O*-digalloylquinic acid, acertannin のラット腹腔内マクロファージホモジネート中での5－リポキシゲナーゼ代謝物 5-HETE およびシクロオキシゲナーゼ代謝物 HHT, thromboxane $B_2$, 6-keto-$PGF_{1\alpha}$ 生成に対する影響について研究を行った。3-*O*-digalloylquinic acid, acertannin, caffeoylmalic acid, caffeoyltartaric acid および rosmarinic acid は選択的に5－リポキシゲナーゼ代謝物 5-HETEの生成を抑制した。上記化合物の 5-HETE 生成に対する 50% 阻害濃度は各々 7.00, 22.5, 425.0, 42.0 および 6.90 $\mu$M であった。$10^{-4}$M～$10^{-5}$M 濃度において，5種のフェノール性化合物はシクロオキシゲナーゼ代謝物 HHT, thromboxane $B_2$ および 6-keto-$PGF_{1\alpha}$ の生成を増加させた。$10^{-3}$M では 3-*O*-digalloylquinic acid は HHT および thromboxane $B_2$ を抑制したが，6-keto-$PGF_{1\alpha}$ の生成を増加させた。

## References

1) Jakschik, B.A., Harper, T., Murphy, R.C.: The 5-lipoxygenase and leukotriene forming enzymes. *Methods in Enzymology*, Lands, W.E.M. and Smith, W.L. (Eds.), Academic Press, New York, **86**, 30-37, 1982.

2) Borgeat, P. and Samuelsson, B.: Arachidonic acid metabolism in polymorphonuclear leukocytes. 5. Unstable intermediate in formation of dihydroxy acids. *Proc. Natl. Acad. Sci. USA* **76**, 3213-3217, 1979.

3) Rädmark, O., Malmsten, C., Samuelsson, B., Goto, G., Marfat, A., Corey, E.J.: Leukotriene A. Isolation from human polymorphonuclear leukocytes. *J. Biol. Chem.* **255**, 11828-11831, 1980.

4) Corey, E.J., Clark, D.A., Goto, G., Marfat, A., Mioskowski, C., Samuelsson, B., Hammarström, S.: Stereospecific total synthesis of a "Slow reacting substance" of anaphylaxis, leukotoriene C-1. *J. Am. Chem. Soc*, **102**, 1436-1439, 1980.

5) Samuelsson, B.: Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science* **220**, 568-575, 1983.

6) Peters, S.P., Siegel, M.I., Kagey-Sobotka, A., Lichtenstein, C.M.: Lipoxygenase products modulate histamine release in human basophils. *Nature* **292**, 455-457, 1981.

7) O'Flaherty, J.T., Thomas, M.J., Hammett, M.J., Carroll, C., McCall, C.E., Wykle, R.L.: 5-L-Hydroxy-6,8,11,14-eicosatetraenoate potentiates the human neutrophil degranulating action of platelet activating factor. *Biochem. Biophys. Res. Comm.* **111**, 1-7, 1983.

8) Morley, J., Bray, M.A., Jones, R.W., Nugsteren, D.H., Van Dorp, D.A.: Prostaglandin and thromboxane production by human and

guinea pig macrophages and leukocytes. *Prostaglandins* **17**, 729-736, 1989.

9) Hamberg, M. and Samuelsson, B. : Thromboxanes; a new group of biologically active compounds derived from prostaglandin endperoxides. *Proc. Natl. Acad. Sci. USA* **72**, 2994-2998, 1975.

10) Moncada, S. and Vane J.R. : Pharmacology and endogenaus roles of prostaglandin endperoxides, thromboxane $A_2$, and prostacyclin. *Pharmac. Rev.* **30**, 293-331, 1979.

11) Moncada, S., Higgs, E.A., Vane J.R. : Human arterial and venous tissues generate prostacyclin (prostagalndin X), a potent inhibitor of platelet aggregation. *Lancet* **1** 18-20, 1977.

12) Okuda, T., Kimura, Y., Yoshida, T., Hatano, T., Okuda, H., Arichi, S. : Studies on the activities of tannins and related compounds from medicinal plants and drugs I. Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. *Chem. Pharm. Bull.* **31**, 1625-1631, 1983.

13) Kimura,Y., Okuda, H., Mori, K., Okuda, T., Arichi, S. : Studies on the activities of tannins and related compounds from medicinal plants and drugs IV. Effects of various extracts of *Geranii* Herba and geraniin on liver injury and lipid metabolism in rats fed peroxidized oil. *Chem. Pharm. Bull.* **32**, 1866-1871, 1984.

14) Kimura, Y., Okuda, H., Okuda, T., Hatano, T., Agata, I., Arichi, S. : Studies on the activities of tannins and related compounds ; V. Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. *Planta Medica* **50**, 473-477, 1984.

15) Kimura, Y., Okuda, H., Okuda, T., Hatano, T., Agata, I., Arichi, S. : Studies on the activities of tannins and related compounds from medicinal plants and drugs VII. Effects of extracts of leaves of *Artemisia* species, and caffeic acid and chlorogenic acid on lipid metabolic injury in rats fed peroxidized oil. *Chem. Pharm. Bull.* **33**, 2028-2034, 1985.

16) Okuda, T., Hatano, T., Ikegami, Y., Chen, L. : Abstract papers, 105 th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April, 1985, p. 454.

17) Okuda, T., Hatano, T., Agata, I., Nishibe, S. : The components of tannic activities in *Labiatae* plants. I. Rosmarinic acid from *Labiatae* plants in Japan. *Yakugaku Zasshi* **106**, 1108-1111, 1986.

18) Okuda, T., Hatano, T., Matsuda, M., Yoshizaki, M. : Abstract papers, 105 th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April, 1985, p. 489.

19) Hatano, T., Hattori, S., Ikeda, Y., Shingu, T., Okuda, T. : Gallotannins having 1,5-anhydro-D-glucitol core and some ellagitannins from *Acer* species. *Chem. Pharm. Bull.* **38**, 1902-1905, 1990.

20) Borgeat, P. and Samuelsson, B. : Transformaton of arachidonic acid by rabbit polymorphonuclear leukocyte ; Formation of a novel dihydroxyeicosatetraenoic acid. *J. Biol. Chem.* **254**, 2643-2646, 1979.

21) Sekiya, K. and Okuda, H. : Selective inhibition of platelet lipoxygenase by baicalein. *Biochem. Biophys. Res. Comm.* **105**, 1090-1095, 1982.

22) Kimura, Y., Okuda, H., Arichi, S., Baba, K., Kozawa, M. : Inhibition of the formation of 5-hydroxy 6,8,11,14 eicosatetraenoic acid from arachidonic acid in polymorphonuclear leukocytes by various coumatins. *Biochim. Biophys. Acta* **834**, 224-229, 1985.

23) Lowry, O.H., Rosebrough, N.J., Farr, A.C., Randall, R.J. : Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.

24) Kakiuchi, N., Hattori, M., Nishizawa, M., Yamagishi, T., Okuda, T., Namba, T. : Studies on dental caries prevention by traditional medicines. VIII. Inhibitory effect of various tannins on glucan synthesis by glucosyltransferase from *Streptococcus mutans*. *Chem. Pharm. Bull.* **34**, 720-725, 1986.

25) Kimura, Y., Okuda, H., Okuda, T., Yoshida, T., Hatano, T., Arichi, S. : Studies on the activities of tannins and related compounds of medicinal plants and drugs. II. Effects of various tannins and related compounds on adrenaline-induced lipolysis in fat cells (1). *Chem. Pharm. Bull.* **31**, 2497-2500, 1983.

26) Kimura, Y., Okuda, H., Okuda, T., Yoshida, T., Hatano, T., Arichi, S. : Studies on the activities of tannins and related compounds of medicinal plants and drugs. III. Effects of various tannins and related compounds on adrenocorticotropic hormone-induced lipolysis and insulin induced lipogenesis from glucose in fat cells (2). *Chem. Pharm. Bull.* **31**, 2501-2506, 1983.

27) Yoshida, T., Mori, A., Hatano, T., Okumura, T., Uehara, I., Komagoe, K., Fujita, Y., Okuda, T. : Studies on inhibition mechanism of autoxidation by tannins and flavonoids. V. Radical-scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picryl radical. *Chem. Pharm. Bull.* **37**, 1919-1921, 1989.

28) Hatano, T., Edamatsu, M., Mori, A., Fujita, Y., Yasuhara, T., Yoshida, T., Okuda, T. : Effects of the interaction of tannins with co-existing substances.VI. Effects of tannins and related polyphenols on superoxide anion radical, and 1,1-diphenyl-2-picryhydrazyl radical. *Chem. Pharm. Bull.* **37**, 2016-2021, 1989.

29) Payne, T.G., Dewald, B., Siegl, H., Gulber, H.U., Ott, H., Baggiolini, M. : Radical scavenging and stimulation of prostaglandin synthesis not antiinflammatory? *Nature* **296**, 160-162, 1982.

30) Fujita, T., Fujimoto, Y., Tanioka, H. : Antioxidant effects on prostaglandin synthesis in rabbit kidney medulla slices. *Experientia* **38**, 1472, 1982.

31) Pace-Asciak, C. : Prostaglandin synthetase activity in the rat stomach fundus. Activation by L-norepinephrine and related compounds. *Biochim. Biophys. Acta* **280**, 161-171, 1972.

32) Pace-Asciak, C. and Nashat, M. : Mechanistic studies on the biosynthesis of 6-ketoprostaglandin $F_{1\alpha}$. *Biochim. Biophys. Acta* **487**, 495-507, 1977.

33) Pace-Asciak, C. : Isolation, structure, and biosynthesis of 6-ketoprostagalndin $F_{1\alpha}$ in the rat stomach. *J. Am. Chem. Soc.* **98**, 2348-2349, 1976.

34) Axelrod, L., Minnich, A.K., Ryan, C.A. : Stimulation of prostacyclin production in isolated rat adipocytes by angiotensin II. vasopressin, and bradykinin : Evidence for two separate mechanisms of prostaglandin synthesis. *Endocrinology* **116**, 2548-2553, 1985.

35) Kimura, Y., Okuda, H., Arichi, S. : Studies on Scutellariae radix XIII ; Effects of various flavonoids on arachidonate metabolism in leukocytes. *Planta Medica* **51**, 143-150, 1985.

36) Hamberg, M., and Samuelsson, B. : Prostaglandin endoperoxides. III. Novel transformations of arachidonic acid in human platelets. *Proc. Natl. Acad. Sci. USA.* **71**, 3400-3404, 1974.

37) Palmer, P.M.J., Stepney, R.J., Higgs, G.A., Eakins, K.E. : Chemokinetic activity of arachidonic acid lipoxygenase products on leukocytes of different species. *Prostaglandins* **20,**, 411-418, 1980.

38) Kimura, Y., Okuda, H., Arichi, S. : Effects of baicalein on leukotriene biosynthesis and degranulation in human polymorphonuclear leukocytes. *Biochim. Biophys. Acta* **922**, 278-286, 1987.

39) Kimura, Y., Okuda, T., Okuda, H. : Effects of flavonoids isolated from licorice roots (*Glycyrrhiza inflata* Bat.) on degranulation in human polymorphonucelar neutrophils. *Phytotherapy Res.* **7**, 335-340, 1993.

40) Asakura, H. and Sasakawa, T. : Recent advances in the treatment of inflammatory bowel diseases. *Igakuno Ayumi* **160**, 895-898, 1992.