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Inhibitory Effect of Glycyrrhetinic Acid Derivatives on Lipoxygenase and Prostaglandin Synthetase

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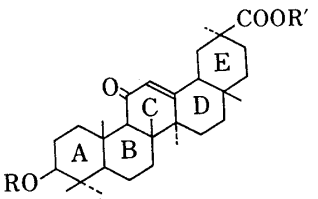
Glycyrrhetinic acid and fifteen derivatives of glycyrrhetinic acid including glycyrrhizin were examined to determine whether they inhibit the lipoxygenase and the cyclooxygenase activities of cloned mastocytoma cells. These compounds were more effective in inhibiting the lipoxygenase than the cyclooxygenase. Of these compounds, the disodium salt of olean-12-ene-3β,30-diol 3β,30-di-*O*-hemiphthalate inhibited 5-lipoxygenase most strongly (ID_{50}, 5.8×10^{-6} M), whereas the half-inhibition dose for the cyclooxygenase was 5.6×10^{-5} M. Glycyrrhetinic acid, the aglycone of glycyrrhizin, slightly inhibited the lipoxygenase and the cyclooxygenase at a concentration of 10^{-4} M but had little effect at 10^{-5} M. Glycyrrhizin and carbenoxolon sodium showed no detectable inhibition of either enzyme at less than 10^{-4} M.

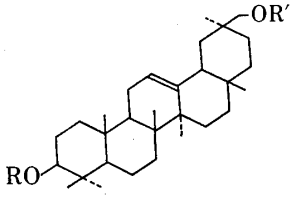
Keywords——glycyrrhizin; glycyrrhetinic acid; carbenoxolon sodium; lipoxygenase; cyclooxygenase; leukotriene; prostaglandin

Glycyrrhizin (Ia), a saponin obtained from the root of licorice (*Glycyrrhizia glabra*) is known to be effective as an anti-inflammatory[2)] and an anti-allergenic agent,[3)] and shows steroid-like action.[4)] Some other effects of glycyrrhizin, such as inhibition of virus growth and inactivation of virus particles,[5)] and interferon-inducing activity[6)] have been found. It has also been reported that glycyrrhetinic acid (Ib), the aglycone of glycyrrhizin, has anti-inflammatory activity in some models of inflammation.[7)] Further, the disodium salt of glycyrrhetinic acid hemiphthalate (Id) derived from glycyrrhetinic acid has been shown to suppress the edema induced by carrageenin, dextran and serotonin.[8)] Glycyrrhetinic acid derivatives of deoxoglycyrrhetol (IIa)[9)] and three kinds of dihemiphthalate groups[10)] (the disodium salt of olean-12-ene-3β,30-diol 3β-30-di-*O*-hemiphthalate (IIg); the disodium salt of olean-9(11), 12-diene-3β,30-diol 3β,30-di-*O*-hemiphthalate (IIIc); the disodium salt of olean-11,13(18)-diene-3β,30-diol 3β,30-di-*O*-hemiphthalate (IVc)) have been shown to prevent experimental gastric ulcer. Clinically, carbenoxolon sodium (Ic) can heal gastric ulcer,[11)] and an ammonium salt of glycyrrhizin combined with cysteine and glycine (Strong Neo-Minophagen C) is administered as a remedy for allergy and chronic hepatitis.[12)]

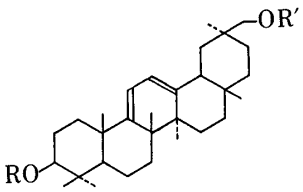
Leukotriene biosynthesis from arachidonic acid begins with the 5-lipoxygenase reaction, leading to 5-hydroperoxyeicosatetraenoic acid (5-HPETE), leukotriene A_4 and 5-hydroxyeicosatetraenoic acid (5-HETE) formation.[13)] Leukotriene B_4 is a very potent chemokinetic and chemotactic agent, which may play an important role in the inflammatory response,[14)] whereas leukotriene C_4, D_4 and E_4 are the active components of the slow reacting substance of anaphylaxis (SRS-A), which is one of the dominant mediators in the bronchoconstriction symptom of allergic asthma.[13)] There are other oxidation products of arachidonic



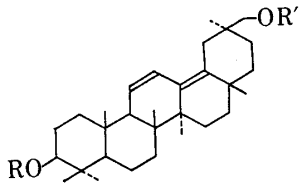
Ia: R=GlcAB(1→2)GlcA
R′=H
Ib: R=H
R′=H
Ic: R=NaOOC(CH$_2$)$_2$CO–
R′=Na
Id: R=–OC(=O)C$_6$H$_4$COONa
R′=Na



IIa: R=H
R′=H
IIb: R=HOOC(CH$_2$)$_2$CO–
R′=H
IIc: R=NaOOC(CH$_2$)$_2$CO–
R′=H
IId: R=NaOOC(CH$_2$)$_2$CO–
R′=NaOOC(CH$_2$)$_2$CO–
IIe: R=–P(=O)(ONa)–ONa
R′=–P(=O)(ONa)–ONa
IIf: R=–OC(=O)C$_6$H$_4$COONa
R′=H
IIg: R=–OC(=O)C$_6$H$_4$COONa
R′=–OC(=O)C$_6$H$_4$COONa



IIIa: R=H
R′=H
IIIb: R=NaOOC(CH$_2$)$_2$CO–
R′=H
IIIc: R=–OC(=O)C$_6$H$_4$COONa
R′=–OC(=O)C$_6$H$_4$COONa



IVa: R=H
R′=H
IVb: R=NaOOC(CH$_2$)$_2$CO–
R′=H
IVc: R=–OC(=O)C$_6$H$_4$COONa
R′=–OC(=O)C$_6$H$_4$COONa

Chart 1. Structures of Glycyrrhetinic Acid Derivatives

acid: the cyclooxygenase pathway leads to prostaglandins, thromboxane and prostacyclin.[15] It is believed that non-steroidal anti-inflammatory drugs inhibit prostaglandin synthesis.[16,17]

Recently, glycyrrhizin has been shown to inhibit prostaglandin E_2 production by macrophages.[18] In this paper, we report that several derivatives of glycyrrhetinic acid have an inhibitory effect on lipoxygenase and cyclooxygenase activities.

Experimental

Assay of 5- and 12-Lipoxygenase and Cyclooxygenase Activities——The enzyme assays were based on the method of Koshihara *et al.*[19] The enzyme used was obtained from cloned mastocytoma P-815, 2-E-6 cells, which were treated with 1 mM *n*-butyrate for 40 h in order to induce cyclooxygenase.[20] The supernatant fraction was prepared from a cell homogenate in 50 mM potassium phosphate buffer (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1% gelatin. Under the standard assay conditions for 5- and 12-lipoxygenase activities, the supernatant derived from cell suspension at 10^7 cells/ml was incubated with 0.2 μCi of [1-^{14}C]arachidonic acid (55.5 Ci/mol), 1.0 mM $CaCl_2$ and 2×10^{-5} M indomethacin at 37 °C for 5 min. For the assay of cyclooxygenase activity, $CaCl_2$ and indomethacin were omitted from the above incubation mixture, and incubation was performed

at 37 °C for 10 min. Both reactions were terminated by adjusting the incubation mixture to pH 3.0 with 1 N HCl. The synthesized HETEs and prostaglandins were extracted with 8 volumes of ethyl acetate, and each extract was concentrated and applied to a silica gel-coated glass plate ($60F_{254}$, layer thickness 0.24 mm, Merck). Thin-layer chromatography was carried out using the following solvent system: ethyl acetate–2,2,4-trimethylpentane–acetic acid–water (11 : 5 : 2 : 10, upper phase). Labeled products separated on the plates were scanned, and radioactive zones were scraped off the plate for measurement of radioactivity in a liquid scintillation spectrometer. The activities of 5- and 12-lipoxygenase and cyclooxygenase were expressed as the sum of radioactivities due to 5-HETE and leukotriene B_4 including 5,12-diHETE; due to 12-HETE and due to synthesized prostaglandin D_2, E_2, $F_{2\alpha}$, respectively.

Inhibitor Treatment——The compounds dissolved in ethyl alcohol of reagent grade (20 μl) and ethyl alcohol (20 μl) were transferred to assay tubes containing [^{14}C]arachidonic acid in toluene. To each tube, one drop of a mixture of propylene glycol–ethyl alcohol (1 : 3) was added. Organic solvents except propylene glycol were evaporated completely under a stream of nitrogen gas. The compounds dissolved in water (20 μl) were directly added to each reaction mixture. All the compounds tested were generous gifts from Prof. S. Shibata, Meiji College of Pharmacy and Mr. N. Nagata, Research Laboratory of Minophagen Co.

Results and Discussion

The effects of the compounds tested on 5- and 12-lipoxygenase and cyclooxygenase activities are summarized in Table I. These compounds were added at 10^{-4} and 10^{-5} M to the cell-free assay system described in the experimental section. At a concentration of 10^{-4} M, the disodium salt of olean-12-ene-3β,30-diol 3β,30-di-*O*-hemiphthalate (IIg) was observed to inhibit the lipoxygenase completely, but glycyrrhizin (Ia) showed no significant inhibition of this enzyme. Glycyrrhetinic acid (Ib) also showed little effect. Ohuchi *et al.*[18] reported that glycyrrhizin (Ia) inhibited prostaglandin E_2 production by activated rat peritoneal macrophage, but its inhibition was not potent. Glycyrrhetinic acid (Ib) did not prevent the synthesis or release of prostaglandins in leucocytes.[21] In our cell-free system, glycyrrhizin (Ia) and glycyrrhetinic acid (Ib) showed very weak inhibition of the cyclooxygenase.

Compound IIg was the strongest inhibitor of lipoxygenase activity among various derivatives of glycyrrhetinic acid tested. The disodium salt of olean-9(11), 12-diene-3β,30-diol 3β,30-di-*O*-hemiphthalate (IIIc) and the disodium salt of olean-11,13(18)-diene-3β,30-diol 3β,30-di-*O*-hemiphthalate (IVc) which has a sodium of hemiphthalate at the 3- and 30-positions of rings A and E in the oleanane skeleton, showed potent inhibition of lipoxygenase, like compound IIg. The sodium salt of olean-12-ene-3β,30-diol 3β-*O*-hemiphthalate (IIf), which has a hemiphthalate at the 3-position of ring A in deoxoglycyrrhetol (IIa), showed potent inhibition but the inhibitory effect of the disodium salt of glycyrrhetinic acid hemiphthalate (Id) was not increased much. Based on the structures of the six tested derivatives, deoxoglycyrrhetol (IIa) showed about 60 percent inhibition of the lipoxygenase. These results suggest that the dihemiphthalate of the oleanane skeleton is necessary for high inhibitory effect on lipoxygenase activity and leukotriene synthesis. The effect of compound IIg on the both enzyme activities was further investigated. 5-Lipoxygenase and cyclooxygenase activities were inhibited dose-dependently, as shown Fig. 1. Their ID_{50} values were 5.8×10^{-6} and 5.6×10^{-5} M for the activities of 5-lipoxygenase and cyclooxygenase, respectively. Its compared with reported inhibitors of 5-lipoxygenase, this compound showed approximately the same potency as esculetin (ID_{50}, 4.0×10^{-6} M)[22] and caffeic acid (ID_{50}, 3.7×10^{-6} M).[23] Carbenoxolon sodium (Ic) and compounds containing four kinds of hemisuccinate groups (IIb,IIc,IIIb,IVb) did not significantly inhibit the lipoxygenase, but the disodium salt of olean-12-ene-3β,30-diol 3β,30-di-*O*-hemisuccinate (IId) was inhibitory. Carbenoxolon sodium (Ic) was found to inhibit the activity of prostaglandin metabolizing enzymes, 15-hydroxyprostaglandin dehydrogenase and Δ^{13}-reductase.[24] Since our results showed that carbenoxolon sodium (Ic) had little effect on the cyclooxygenase activity, it might affect prostaglandin biosynthesis indirectly.

TABLE I. Inhibition of Lipoxygenase and Cyclooxygenase Activities by Glycyrrhetinic Acid Derivatives

Compound	Conc. (M)	Lipoxygenase		Cyclooxygenase
		5-Lip.	12-Lip.	
Ia	10^{-4}	14	16	5
	10^{-5}	—	—	—
Ib	10^{-4}	32	43	26
	10^{-5}	19	8	—
Ic	10^{-4}	3	10	16
	10^{-5}	—	—	—
Id	10^{-4}	45	68	23
	10^{-5}	11	40	—
IIa	10^{-4}	55	64	25
	10^{-5}	—	—	—
IIb	10^{-4}	27	54	—
	10^{-5}	—	—	—
IIc	10^{-4}	—	—	10
	10^{-5}	—	—	—
IId	10^{-4}	55	75	47
	10^{-5}	10	37	—
IIe	10^{-4}	45	62	30
	10^{-5}	29	40	—
IIf	10^{-4}	83	90	8
	10^{-5}	22	29	—
IIg	10^{-4}	97	100	60
	10^{-5}	62	69	21
IIIa	10^{-4}	—	18	—
	10^{-5}	—	—	—
IIIb	10^{-4}	—	—	36
	10^{-5}	—	—	—
IIIc	10^{-4}	91	96	48
	10^{-5}	34	36	17
VIa	10^{-4}	45	46	34
	10^{-5}	31	35	—
VIb	10^{-4}	31	20	—
	10^{-5}	—	—	—
VIc	10^{-4}	94	85	71
	10^{-5}	28	36	25

Values of inhibition are expressed as percent of the control. Similar results were obtained in three separate experiments.

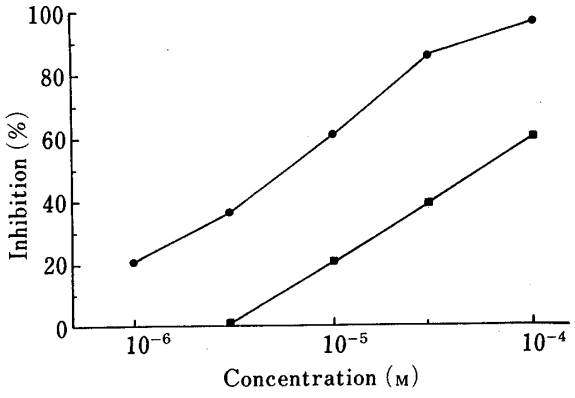


Fig. 1. Effects of Compound IIg on 5-Lipoxygenase and Cyclooxygenase Activities

Various concentrations of compound IIg were added to the cell-free assay system for measuring lipoxygenase and cyclooxygenase activities as described in the experimental section.
●, 5-lipoxygenase; ■, cyclooxygenase.

We found that derivatives of glycyrrhetinic acid have inhibitory activity toward both enzymes. The mechanism of inhibition is obscure, but the active site of lipoxygenase is presumably inactivated by the hemiphthalate groups of oleanane-type triterpenoids.

5-Lipoxygenase is the first enzyme involved in the biosynthesis of several leukotrienes related to asthma, allergic disease and inflammation.[13] 12-HPETE formed by 12-lipoxygenase was recognized to be able to activate 5-lipoxygenase in circulating leucocytes and lung macrophages, with subsequent release of bronchoconstricting leukotrienes.[25] Therefore, compounds containing hemiphthalate groups may be available as inhibitors of leukotriene biosynthesis. It is possible that these compounds could be developed as drugs to treat inflammation and allergic disease by suppressing lipoxygenase, cyclooxygenase and phospholipase A_2 activities, since a previous study showed that glycyrrhizin inhibited phospholipase A_2.[26]

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References and Notes

1) Present address: *Faculty of Dentistry, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo 113, Japan.*
2) S. Sotomatsu, Y. Takaishi, J. Hiroi, A. Namikata, and N. Okano, *Skin & Urology*, **21**, 138 (1959).
3) T. Kuroyanagi and M. Saito, *Arerugi*, **15**, 67 (1966).
4) A. Kumagai, S. Yano, M. Otomo, and K. Takeuchi, *Endocrinol. Jpn.*, **4**, 17 (1957).
5) R. Pompei, O. Flore, M. Antonietta, M. A. Pani, and B. Loddo, *Nature* (London), **281**, 689 (1979).
6) Y. Hayashi, K. Nakata, H. Aso, F. Suzuki, and N. Ishida, *Yakuri To Chiryo*, **7**, 3861 (1979).
7) R. S. H. Finney and A. L. Tarnoky, *J. Pharm. Pharmacol.*, **12**, 49 (1960).
8) S. Tomizawa and Y. Hara, *Pharmacometrics*, **11**, 677 (1976).
9) K. Takahashi, S. Shibata, S. Yano, M. Harada, H. Saito, Y. Tamura, and A. Kumagai, *Chem. Pharm. Bull.*, **28**, 3349 (1980).
10) S. Yano, K. Nakamaru, K. Watanabe, M. Harada, K. Takahashi, S. Shibata, N. Nagata, and K. Hirabayashi, Abstracts of Papers, 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April 1985, p. 155.
11) R. Doll, I. D. Hill, C. Hutton, and D. J. Underwood, *Lancet*, II, 793 (1962).
12) H. Suzuki, *Wakan-yaku*, Proc. Symp., **12**, 114 (1979).
13) B. Samuelsson, *Science*, **220**, 568 (1983).
14) A. W. Ford-Hutchinson, M. A. Bray, M. V. Doig, M. E. Shipley, and M. J. H. Smith, *Nature* (London), **286**, 264 (1980).
15) B. Samuelsson, M. Goldyne, E. Granström, M. Hamberg, S. Hammarstöm, and C. Malmsten, *Ann. Rev. Biochem.*, **47**, 997 (1978).
16) J. R. Vane, *Nat. New Biol.*, **231**, 232 (1971).
17) C. Pace-Asciak and S. Cole, *Experientia*, **31**, 143 (1975).
18) K. Ohuchi, Y. Kamada, L. Levine, and S. Tsurufuji, *Prostaglandins & Medicine*, **7**, 457 (1981).
19) Y. Koshihara, M. Mizumura, and S. Murota, *J. Biol. Chem.*, **257**, 7302 (1982).
20) Y. Koshihara, M. Kawamura, T. Senshu, and S. Murota, *Biochem. J.*, **194**, 111 (1981).
21) F. Capasso, N. Mascolo, G. Autore, and M. R. Duraccio, *J. Pharm. Pharmacol.*, **35**, 332 (1983).
22) T. Neichi, Y. Koshihara, and S. Murota, *Biochim. Biophys. Acta*, **753**, 130 (1983).
23) Y. Koshihara, T. Neichi, S. Murota, A. Lao, Y. Fujimoto, and T. Tatsuno, *Biochim. Biophys. Acta*, **792**, 92 (1984).
24) B. M. Peskar, A. Holland, and B. A. Peskar, *J. Pharm. Pharmacol.*, **28**, 146 (1976).
25) J. Maclouf, B. F. DeLacols, and P. Borgeat, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 6042 (1982).
26) E. Okimasu, Y. Moromizato, S. Watanabe, J. Sasaki, N. Shiraishi, Y. M. Morimoto, M. Miyahara, and K. Utsumi, *Acta Med. Okayama*, **37**, 385 (1983).