Modulation by glycyrrhetic acid derivatives of TPA-induced mouse ear oedema

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1 The anti-inflammatory effects of glycyrrhetic acid and its derivatives on TPA (12-O-tetradecanoylphorbol-13-acetate)-induced mouse ear oedema were studied. The mechanisms of TPA-induced ear oedema were first investigated with respect to the chemical mediators.

2 The formation of ear oedema reached a maximum 5 h after TPA application (2 µg per ear) and the prostaglandin E₂ (PGE₂) production of mouse ear increased with the oedema formation.

3 TPA-induced ear oedema was prevented by actinomycin D and cycloheximide (0.1 mg per ear, respectively) when applied during 60 min after TPA treatment.

4 Of glycyrrhetic acid derivatives examined, dihemipthalate derivatives (IIe, IIe', IIIa, IIIa', IVa, IVa') most strongly inhibited ear oedema on both topical (ID₅₀, 1.6 mg per ear for IIe, 2.0 mg per ear for IIIa and 1.6 mg per ear for IVa) and oral (ID₅₀, 88 mg kg⁻¹ for IIe', 130 mg kg⁻¹ for IIIa' and 92 mg kg⁻¹ for IVa') administration.

5 Glycyrrhetic acid (Ia) and its derivatives applied 30 min before TPA treatment were much more effective in inhibiting oedema than when applied 30 min after TPA. A dihemipthalate of triterpenoid compound IVa completely inhibited oedema, even when applied 3 h before TPA treatment.

6 Glycyrrhetic acid (Ia) and deoxglycyrrhetol (IIa), the parent compounds, produced little inhibition by oral administration at less than 200 mg kg⁻¹.

7 These results suggest that the dihemipthalate derivatives of triterpenes derived from glycyrrhetic acid by chemical modification are useful for the treatment of skin inflammation by both topical and oral application.

Introduction

Glycyrrhizin is a major saponin of liquorice root (*Glycyrrhiza* spp.), which has been reported to have anti-allergic (Kuroyanagi & Saito, 1966), ocular anti-inflammatory (Tanaka *et al.*, 1987) and interferon-inducing (Shinada *et al.*, 1986) activities as well as anti-viral effects (Pompei *et al.*, 1979) including those against varicella-herpes zoster virus (VZV) and human T-cell lymphotropic virus (HTLV-III) (AIDS virus) (Ito *et al.*, 1987). Its aglycone, glycyrrhetic acid (Ia), also has pharmacological activities as an anti-inflammatory (Finney & Somers, 1958; Capasso *et al.*, 1982) and anti-tumour promoter agent (Nishino *et al.*, 1986), as well as inhibiting growth of

mouse melanoma (Abe *et al.*, 1987) and inhibiting communication across intercellular gap junctions (Davidson *et al.*, 1986). Some glycyrrhetic acid derivatives have been prepared in an attempt to enhance the therapeutic activity and suppress the unfavourable side-effect (pseudo-aldosteronism) of the parent compound (Takahashi *et al.*, 1980; Shibata *et al.*, 1987). Clinically, carbenoxolone sodium (glycyrrhetic acid 3-O-hemisuccinate sodium salt) has been used to treat gastric ulcer (Doll *et al.*, 1962). Of the derivatives of glycyrrhetic acid, three kinds of dihemipthalate compounds (disodium salt of 18β-olean-12-ene-3β,30-diol di-O-hemipthalate (IIe), 18β-olean-9(11),12-diene-3β,30-diol di-O-hemipthalate (IIIa), olean-11,13(18)-diene-3β,30-diol di-O-hemipthalate (IVa)) have

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been found to inhibit experimental gastric ulcer (Yano & Watanabe, unpublished). Furthermore, we have reported that these dihemiphthalate compounds inhibit lipoxygenase and cyclo-oxygenase activities in a cell-free system from cloned mastocytoma cells (Inoue *et al.*, 1986) and show antinociceptive and anti-type IV allergic effects (Inoue *et al.*, 1987). Recently, these compounds have been found to inhibit arachidonic acid-induced mouse ear oedema which is mainly mediated by arachidonic acid metabolites such as leukotriene C_4 (LTC_4) and prostaglandin E_2 (PGE_2) (Inoue *et al.*, 1988).

Mouse ear oedema induced by croton oil and its irritant principle, TPA (12-O-tetradecanoylphorbol-13-acetate), has been used as a test for anti-inflammatory activity (Carlson *et al.*, 1985; Bird *et al.*, 1986; Tubaro *et al.*, 1986), being suppressed by non-steroidal and steroidal anti-inflammatory agents. In addition, lipoxygenase inhibitors and dual inhibitors of lipoxygenase/cyclo-oxygenase also inhibited TPA-induced ear oedema (Young *et al.*, 1983; Carlson *et al.*, 1985). However, the mechanism involved in the induction of this type of oedema has not been completely elucidated even though TPA-induced ear oedema was well-characterized by Young *et al.* (1983). In the present paper we measured the PGE_2 and LTC_4 content of TPA-treated mouse ears and investigated the contribution of new protein synthesis to the oedema response. In addition, we investigated the inhibitory effect of glycyrrhetic acid derivatives on the oedema response.

Methods

Assay for TPA-induced ear oedema

Six week old male ddY mice (Shizuoka Laboratory Animal Center, Japan) were assigned to eight treatment groups, housed together and acclimatized under standard conditions for one week with free access to food and water. 12-O-Tetradecanoylphorbol-13-acetate (TPA, Sigma Chemical Co., U.S.A.) was dissolved in acetone at a concentration of $100 \mu\text{g ml}^{-1}$. Each mouse was then treated with $20 \mu\text{l}$ ($2 \mu\text{g}$ per ear) of TPA on both surfaces of an ear. Test compounds were dissolved in acetone or ethanol for topical application, and were suspended with 1% polyoxyethylene sorbitan monooleate (Tween 80, Tokyo Kasei Chemical Industry, Japan) in saline (0.9% NaCl) for oral administration. Both treatments were given 30 min before and/or after TPA application. Control mice received the vehicle only. Ear oedema was measured 5 h after TPA application with dial calipers (Ozaki Factory,

Japan) measuring to an accuracy of 0.01 mm. Statistical significance was assessed by Student's *t* test.

Determination of LTC_4 and PGE_2 in ear oedema

Animals treated with TPA were killed at designated intervals, and each ear was removed immediately and stored on dry-ice. It was homogenized with ice-cold 80% ethanol for LTC_4 and 95% ethanol for PGE_2 by a motor driven homogenizer (5,000 r.p.m. 30 s). The homogenate was centrifuged at 4,500 *g* for 15 min at 4°C. LTC_4 in 80% ethanol extract or a further purified fraction by Sep-Pac C_{18} cartridge was measured by LTC_4 -radio-immunoassay (New England Nuclear, Boston, MS, U.S.A.). PGE_2 in ethanol was absorbed by octadecylsilyl silica (ODS) and then washed with ethanol/0.05 N HCl and petroleum ether to remove protein and liquid. Finally, PGE_2 was eluted with ethyl acetate. The eluate was further purified by a silica minicolumn (BOND ELUT Si) with the following eluate solvent: acetonitrile-chloroform-acetic acid (20:80:0.5). PGE_2 was measured by radio-immunoassay (New England Nuclear, U.S.A.). The recovery of LTC_4 from the 80% ethanol-homogenate and that of PGE_2 from 95% ethanol were 79% and 97.5%, respectively.

Materials

Glycyrrhetic acid and its derivatives were prepared according to Shibata *et al.* (1987). Other compounds were purchased as follows: aspirin from Nakarai Chemical Co., Japan; nordihydroguaiaretic acid (NDGA), indomethacin, cyproheptadine and dexamethasone from Sigma Chemical Co., U.S.A.; actinomycin D and cycloheximide from Sigma Chemical Co., U.S.A. AA 861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone) was a generous gift from Takeda Chemical Industry Co., Japan.

Results

The ear oedema induced by a single topical application of $2 \mu\text{g}$ TPA was examined at various intervals by measuring ear thickness. As found previously (Young *et al.*, 1983), the ear thickness had increased 3 h after TPA application and reached a maximum at 5 h (Figure 1). Oedema then decreased gradually but swelling still remained 24 h after TPA application and resulted in eventual necrosis. The level of endogenous PGE_2 began to increase 1 h after TPA

Figure 1 The time course of ear oedema formation and chemical mediator production induced by TPA. Mouse ears were treated by topical application of TPA (2 µg). The measurement of prostaglandin E₂ (PGE₂) and leukotriene C₄ (LTC₄) was as described in Methods. Each point represents the mean value from 8 animals; vertical lines show s.e.mean. Ear oedema is the mean ear thickness of test animals minus that of control animals. (●) Ear thickness; (○) PGE₂; (▲) LTC₄.

application and continued for a further 4 h in accordance with the oedema formation, whereas LTC₄ production did not increase 5 h after application (Figure 1), and LTB₄ in the ear treated with TPA was not detected by radio-immunoassay. Since TPA is known to induce DNA synthesis (Hennings & Boutwell, 1970) and activate ornithine decarboxylase (ODC) (O'Brien *et al.*, 1975), the role of RNA and newly synthesized protein in development of

Figure 2 Inhibitory effects of cycloheximide (●) and actinomycin D (▲) on TPA-induced ear oedema. These compounds (0.1 mg per ear) were applied topically at various times before or after TPA treatment. Ear thickness was measured 5 h after TPA application. Each point represents the mean from 10 animals; vertical lines show s.e.mean. ** $P < 0.01$, *** $P < 0.001$ compared by paired Student's *t* test with the appropriate TPA control groups (○ and △).

oedema was investigated by use of inhibitors applied topically. Figure 2 shows that TPA-induced ear oedema was significantly inhibited by either actinomycin D or cycloheximide (each at a dose of 0.1 mg per ear) when applied during the first 60 min after TPA application. These compounds were particularly effective when applied immediately after TPA (64% inhibition, $P < 0.001$), suggesting that the new protein synthesis was induced immediately after TPA application.

The anti-inflammatory effects of glycyrrhetic acid (Ia) and its derivatives (for structures see Figure 3) were examined by topical and oral application in the ear oedema model and the results are summarized in Table 1. The compounds were tested 30 min before or after TPA treatment at a dose of 1 mg per ear. Dexamethasone (0.1 mg per ear) was included as the positive control. With the exception of compounds IIb and IId, glycyrrhetic acid (Ia) and its analogues were very effective, inhibiting oedema by 80% ($P < 0.001$) when applied 30 min before TPA treatment. Of these active compounds the three dihemiphthalate compounds, IIe, IIIa and IVa, inhibited oedema most effectively when applied to the ear before TPA: their effects decreased to 40% ($P < 0.001$) when applied after TPA. Similarly, NDGA was marginally more effective when applied before, rather than following, application of TPA. AA 861, aspirin and cyproheptadine were modest inhibitors, whereas dexamethasone was very effective when applied after TPA.

The dose-dependence of the antioedema effect was studied and IIe, IIIa and IVa, were found to have ED₅₀ values of 1.6 mg per ear, 2.0 mg per ear, and 1.6 mg per ear, respectively (Figure 4). In contrast, NDGA, aspirin and cyproheptadine did not inhibit oedema in a dose-dependent fashion (data not shown). Compound IVa, a strong inhibitor of oedema, was then applied topically (1 mg per ear) to the ear at various times either before or after TPA application. Figure 5 shows that compound IVa inhibited ear oedema when applied before TPA treatment, but its activity decreased markedly when it was administered following TPA application.

The water soluble disodium salts of the hemiphthalates (Ic', IId', IIe', IIIa' and IVa') were given orally 30 min after TPA treatment. Compounds IIe', IIIa' and IVa' inhibited ear oedema dose-dependently with ED₅₀ values of 88 mg kg⁻¹, 130 mg kg⁻¹ and 92 mg kg⁻¹, respectively. Glycyrrhetic acid 3-O-hemiphthalate (Ic') also showed a slight inhibition ($P < 0.05$) at a dose of 200 mg kg⁻¹, but had little effect at less than 100 mg kg⁻¹. In contrast, glycyrrhetic acid (Ia) and deoxglycyrrhetol (IIa) were inactive by the oral route even when applied 30 min before TPA treatment (data not shown).

Figure 3 The structures of glycyrrhetic acid derivatives.

Discussion

The application of TPA to mouse skin leads to oedema, cellular infiltration and skin tumour promotion (Diamond *et al.*, 1980; Blumberg *et al.*, 1981). Young *et al.* (1983) suggested the participation of arachidonate metabolites in the oedema formation caused by TPA, and TPA has been shown to induce PGE_2 production from epidermal cells (Bresnick *et al.*, 1979). We have now demonstrated that TPA induces PGE_2 production by the cells of the ear, and PGE_2 content increased with the same time course as oedema formation (Figure 1). In contrast, LTC_4 did not increase and LTB_4 was not detected. Since cyclo-oxygenase inhibitors such as indomethacin, aspirin and naproxen have been shown to suppress TPA-induced ear oedema (Young *et al.*, 1983;

Carlson *et al.*, 1985; present results), PGE_2 is presumably a mediator of TPA-induced oedema. It is also probable that histamine and 5-hydroxytryptamine contribute to oedema since cyproheptadine was inhibitory. Since TPA did not stimulate increased levels of lipoxigenase products LTB_4 and LTC_4 , the lipoxigenase inhibitors AA 861 and NDGA may have inhibited oedema formation by virtue of their weak cyclo-oxygenase-inhibitory activity or some other, unknown, property.

It is known that application of TPA can induce changes in the epidermal protein pool which are prevented by anti-inflammatory steroids and cycloheximide (Slaga & Nelson, 1984). We have now shown that the oedema induced by TPA is strongly

Table 1 Inhibition (%) of TPA-induced mouse ear oedema by glycyrrhetic acid derivatives administered either orally or topically

Compound	Topical inhibition (%)			Oral inhibition (%)	
	Dose (mg per ear)	Pre-treat	Post-treat	Dose (mg per kg)	Post-treat
Ia	1	81***	24**	200	8
Ib	1		21*	200	5
Ic (c')	1	88***	23***	100	9
				200	40*
IIa				200	0
IIb	1	48**	25***	200	2
IIc	1			200	10
II'd (d')	1	49***	20***	200	15
IIe (e')	1	93***	40***	25	10
				50	28*
				100	54**
				150	96***
IIIa (a')	1	91***	41***	25	1
				50	28**
				100	43**
				150	78***
IVa (a')	1	96***	48***	25	18
				50	29*
				100	71***
				150	93***
NDGA	1	38***	24**		
AA 861	1	19**	30**		
Aspirin	1	8	16**		
Indomethacin	1	32**	31**		
Cyproheptadine	1	0	35**		
Dexamethasone	0.1		83**		

Compounds were applied 30 min before and after TPA treatment.

Oral application was performed 30 min after TPA treatment.

Statistical significance from the control at * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ ($n = 8$). For further details, see text.

Figure 4 The dose-dependent inhibition of dihemipthalate compounds on TPA-induced ear oedema. Various doses of the compounds were applied topically to the ear 30 min after TPA treatment. Each point represents mean percentage inhibition compared with control for 8 animals. (●) Compound IIe; (▲) compound IIIa; (○) compound IVa.

inhibited by dexamethasone, actinomycin D and cycloheximide. Therefore, it is likely that new protein synthesis induced by TPA contributes to the oedema response.

Among the glycyrrhetic acid derivatives examined on TPA-induced ear oedema, compounds (IIe, IIe', IIIa, IIIa', IVa and IVa') with hemipthalate groups at the 3- and 30-positions of ring A and E in the oleanane skeleton were found to inhibit ear oedema most strongly whether administered topically or orally. Glycyrrhetic acid (Ia) was weakly effective when given topically but orally this compound and deoxoglycyrrhetol (IIa), the parent compounds of the dihemipthalate derivatives, had little effect. Glycyrrhetic acid hemipthalate (Ic') and deoxoglycyrrhetol 3-O-monohephtalate (II'd') also had little effect. These results suggest that the dihemipthalate substitution on the oleanane skeleton of these compounds is essential for the potent inhibitory effect on TPA-induced ear oedema. This coincides with the results obtained from biochemical

Figure 5 The time effect for topical application of compound IVa on TPA-induced ear oedema. The compound was applied at various times before and after TPA treatment. Ear thickness was determined 5 h after TPA treatment. Each point represents mean percentage inhibition compared with control for 8 animals. The statistically significant difference from control indicated by ** $P < 0.01$ and *** $P < 0.001$.

and pharmacological studies on the inhibitory effect of these compounds on lipoxygenase and cyclo-oxygenase activities (Inoue *et al.*, 1986), on the analgesic and the anti-type IV allergic effect (Inoue *et al.*, 1987) and on the inhibition of arachidonic acid-induced mouse ear oedema (Inoue *et al.*, 1988).

It is noticeable that the topical inhibitory effect of glycyrrhetic acid and its derivatives was more potent when they were applied 30 min before TPA treatment than when applied 30 min after TPA. Particularly, compound IVa showed more than 90% inhibition when applied up to 3 h before TPA treatment. This characteristic suggests that modulation of oedema by the dihemipthalate compounds could be

due to interaction of the compounds with the intracellular receptor for TPA, protein kinase-C. These compounds were more effective in inhibiting 5-lipoxygenase than cyclo-oxygenase which was suppressed about 20% in cloned mastocytoma cells at 10^{-5} M (Inoue *et al.*, 1986). In addition, we have demonstrated that these dihemipthalate compounds inhibit arachidonic acid-induced mouse ear oedema by suppressing the production of LTC_4 and PGE_2 (Inoue *et al.*, 1988). However, glycyrrhetic acid analogues could not inhibit PGE_2 production in TPA-induced mouse ear oedema when given topically (2 mg per ear) or orally (100 mg per ear) 30 min after TPA treatment, whereas indomethacin (1 mg per ear) completely suppressed PGE_2 formation (data not shown). Thus the dihemipthalate derivatives of glycyrrhetic acid analogues appear to have anti-inflammatory effects by a mechanism(s) in addition to inhibition of arachidonate metabolism. It has been shown previously that the dihemipthalate compounds inhibit vascular permeability and type IV allergy (Inoue *et al.*, 1987) and protect the gastric mucosa in an experimental model of gastric ulceration (Yano & Watanabe, unpublished), the mechanism of which is being studied. Thus, the dihemipthalate compounds described have an interesting profile of action and may be of value for the treatment of inflammatory skin diseases.

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References

- ABE, H., OHYA, N., YAMAMOTO, K.F., SHIBUYA, T., ARICHI, S. & ODASHIMA, S. (1987). Effects of glycyrrhizin and glycyrrhetic acid on growth and melanogenesis in cultured B 16 melanoma cells. *Eur. J. Cancer Clin. Oncol.*, **23**, 1549–1555.
- BIRD, J., KIM, H.P. & LEE, H.J. (1986). Topical anti-inflammatory activity of esters of steroid 21-oic acid. *Steroids*, **47**, 35–40.
- BLUMBERG, P.M. (1981). In vitro studies in the mode of action of the phorbol ester potent tumor promoters. *CRC Crit. Rev. Toxicol.*, **9**, 199–234.
- BRESNICK, E., MEUNIER, P. & LAMDEN, M. (1979). Epidermal prostaglandins after topical application of a tumor promoter. *Cancer Letter*, **7**, 121–125.
- CAPASSO, F., MASCOLO, N., AUTORE, G. & DURACCIO, M.R. (1983). Glycyrrhetic acid, leucocytes and prostaglandins. *J. Pharm. Pharmacol.*, **35**, 332–335.
- CARLSON, R.P., O'NEILL-DAVIS, L., CHANG, J. & LEWIS, A.J. (1985). Modulation of mouse ear edema by cyclo-oxygenase and lipoxygenase inhibitors and other pharmacologic agents. *Agents & Actions*, **17**, 197–204.
- DAVIDSON, J.S., BAUMGARTEN, I.M. & HARLEY, E.H. (1986). Reversible inhibition of intercellular junctional communication by glycyrrhetic acid. *Biochem. Biophys. Res. Commun.*, **134**, 29–36.
- DIAMOND, L., O'BRIEN, T.J. & BAIRD, W.M. (1980). Tumor promoters and the mechanism of tumor promotion. *Adv. Cancer Res.*, **32**, 1–74.
- DOLL, R., HILL, D., HUTTON, C. & UNDERWOOD, D.J. (1962). Clinical trial of a triterpenoid liquorice compound in gastric and duodenal ulcer. *Lancet*, **ii**, 793–796.
- FINNEY, R.S.H. & SOMERS, G.F. (1958). The anti-inflammatory activity of glycyrrhetic acid and derivatives. *J. Pharm. Pharmacol.*, **10**, 613–620.
- HENNINGS, H. & BOUTWELL, R.K. (1970). Studies on the mechanism of skin tumor production. *Cancer Res.*, **30**, 312–320.

- INOUE, H., SAITO, H., KOSHIHARA, Y. & MUROTA, S. (1986). Inhibitory effect of glycyrrhetic acid derivatives on lipoxygenase and prostaglandin synthetase. *Chem. Pharm. Bull. (Tokyo)*, **34**, 897-901.
- INOUE, H., MORI, T., SHIBATA, S. & SAITO, H. (1987). Pharmacological activities of glycyrrhetic acid derivatives: Analgesic and anti-type IV allergic effect. *Chem. Pharm. Bull. (Tokyo)*, **35**, 3888-3893.
- INOUE, H., MORI, T., SHIBATA, S. & KOSHIHARA, Y. (1988). Inhibitory effect of glycyrrhetic acid derivatives on arachidonic acid-induced mouse ear oedema. *J. Pharm. Pharmacol.*, **40**, 272-277.
- ITO, M., NAKASHIMA, H., BABA, M., PAUWELS, R., DE CLERCQ, E., SHIGETA, S. & YAMAMOTO, N. (1987). Inhibitory effect of glycyrrhizin on the in vitro infectivity and cytopathic activity of human immunodeficiency virus [HIV (HTLV-III/LAV)]. *Antiviral Res.*, **7**, 127-137.
- KUROYANAGI, T. & SAITO, M. (1966). Effect of prednisolone and glycyrrhizin on passive transfer of experimental allergic encephalomyelitis. *Allergy*, **15**, 67-75.
- NISHINO, H., YOSHIOKA, K., IWASHIMA, A., TAKIZAWA, H., KONISHI, S., OKAMOTO, H., OKABE, H., SHIBATA, S., FUJIKI, H. & SUGIMURA, T. (1986). Glycyrrhetic acid inhibits tumor-promoting activity of teleocidin and 12-O-tetradecanoylphorbol-13-acetate in two-stage mouse skin carcinogenesis. *Jpn. J. Cancer Res.*, **77**, 33-38.
- O'BRIEN, T.G., SIMSIMAN, R.C. & BOUTWELL, R.K. (1975). Induction of polyamine-biosynthetic enzymes in mouse epidermis by tumor promoting agents. *Cancer Res.*, **35**, 1662-1670.
- POMPEI, R., FLORE, O., MARCCIALIS, M.A., PANI, A. & LODDO, B. (1979). Glycyrrhizic acid inhibits virus growth and inactivates virus particles. *Nature*, **281**, 689-690.
- SHIBATA, S., TAKAHASHI, K., YANO, S., HARADA, M., SAITO, H., TAMURA, Y., KUMAGAI, A., HIRABAYASHI, K., YAMAMOTO, M. & NAGATA, N. (1987). Chemical modification of glycyrrhetic acid in relation to the biological activities. *Chem. Pharm. Bull. (Tokyo)*, **35**, 1910-1918.
- SHINADA, M., AZUMA, M., KAWAI, H., SAZAKI, K., YOSHIDA, I., YOSHIDA, T., SUZUTANI, T. & SAKUMA, T. (1986). Enhancement of interferon- γ production in glycyrrhizin-treated human peripheral lymphocytes in response to concanavalin A and to surface antigen of hepatitis B virus (42241). *Proc. Soc. Exp. Biol. Med.*, **181**, 205-210.
- SLAGA, T.J. & NELSON, K.G. (1984). Critical protein modifications during skin tumor promotion in mice. In *Mechanism of Tumor Promotion*, Vol 2. ed. Slaga, T.J. pp. 159-187. Florida: CRC Press Inc.
- TAKAHASHI, K., SHIBATA, S., YANO, S., HARADA, M., SAITO, H., TAMURA, Y. & KUMAGAI, A. (1980). Chemical modification of glycyrrhetic acid in relation to the biological activities. *Chem. Pharm. Bull. (Tokyo)*, **28**, 3449-3452.
- TANAKA, H., HASEGAWA, T., MATSUSHITA, M., MIICHI, H. & HAYASHI, S. (1987). Quantitative evaluation of ocular anti-inflammatory drugs based on measurements of corneal temperature in rabbits: Dexamethasone and glycyrrhizin. *Ophthalmic Res.*, **19**, 213-220.
- TUBARO, A., DRI, P., MELATO, M., MULAS, G., BIANCHI, P., DEL NEGRO, P. & DELLA LOGGIA, R. (1986). In the croton oil ear test the effects of non-steroidal anti-inflammatory drugs (NSAIDS) are dependent on the dose of the irritant. *Agents & Actions*, **19**, 371-373.
- YOUNG, J.M., WAGNER, B.M. & SPIRES, D.A. (1983). Tachyphylaxis in 12-O-tetradecanoylphorbol acetate- and arachidonic acid-induced ear edema. *J. Invest. Dermatol.*, **80**, 48-52.

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