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Effect of synthesized cyclohexanol derivatives using L-menthol as a lead compound on the percutaneous absorption of ketoprofen

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Abstract

L-Menthol was selected as a lead compound to synthesize new candidates for percutaneous absorption enhancers. In a previous study, *O*-ethylmenthol (MET) was the most effective compound and caused relatively little skin irritation. To develop more effective compounds, mono- or disubstitute groups of cyclohexane with an *O*-ethyl group were synthesized. Some 35 compounds were synthesized and evaluated for their promoting activity and effect on skin. An in vivo percutaneous absorption study was performed using rats with hydrogel containing ketoprofen and each of the synthesized compounds. The plasma concentration of ketoprofen was determined after the application of hydrogel to the abdominal area of rats. The apparent penetration rate (R_p) was estimated based on the pharmacokinetic model with a constant rate of penetration through the skin after the lag time. The 2-compartment model was applied to the data obtained from the iv administration. As an index to evaluate the promoting activity of each enhancer, an enhancement factor (E_f) was defined as follows: $E_f = R_p$ (with enhancer)/R_p (without enhancer). Irritation to skin was pathologically evaluated. The treated area of rat abdominal skin was exised after the in vivo experiment using total irritation score (TIS). The compound having a C-3 positioned *iso*-butyl group on the chemical structure was the most effective and caused relatively little irritation among mono-substituted compounds. In the case of di-substituted compounds, all had the same effect as or a stronger effect than MET. Furthermore, the promoting activity almost corresponded to irritation. To estimate log *P*, one of the physicochemical properties of molecules, a computer program 'CAChe' was employed. The log *P* was calculated using the atom typing scheme. Multiple regression analysis revealed that the relations between E_f or TIS and log *P* were parabolic. It was suggested that the optimum log *P* value reflects the promoting activity to enhance percutaneous absorption of ketoprofen.

Keywords: Cyclohexanol derivatives; L-Menthol; Lead compound; Percutaneous absorption enhancer; Ketoprofen

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1. Introduction

Transdermal drug delivery is an effective method to deliver drugs systemically. However, the barriability of the stratum corneum to foreign substances must be overcome. To achieve this, many chemical and physical promoting methods have been developed. In studies on chemical promoting methods, cyclic monoterpenes, such as D-limonene or L-menthol, were found to be effective in inducing percutaneous absorption of indomethacin (Okabe et al., 1989).

Recently, *O*-alkylmenthol and *O*-acylmenthol derivatives were synthesized and their promoting activity for the percutaneous absorption of ketoprofen from hydrogel in rats was evaluated (Negishi et al., 1995; Nakamura et al., 1996). *O*-Ethylmenthol (MET) was the most effective and caused relatively little skin irritation. It was considered that MET partitioned the hydrophobic region in the stratum corneum and increased the permeability of the drugs. Okusa et al. reported that the excretion of urine was retained until 8 h after administration of oxybutin hydrogel containing 0.5% MET (Okusa et al., 1997). It was also reported that MET was effective against not only acidic drugs such as ketoprofen but also basic drugs. Furthermore, thiomenthol derivatives were synthesized and investigated their effect using ketoprofen (Takanashi et al., 1999). In a series of thiomenthol derivatives, the compounds exhibiting strong enhancement action caused severe skin damage.

In this study, L-menthol was selected as a lead compound to synthesize new candidates for percutaneous absorption enhancers. Some 35 compounds were newly synthesized and evaluated as to their promoting activity and effect on skin. In a previous study, it was revealed that MET showed strong efficiency at very low concentrations when added to hydrogel. To develop more effective compounds, mono- or di-substitute groups of cyclohexanol with an *O*-ethyl group were synthesized. Their enhancement activity for percutaneous absorption of ketoprofen from hydrogel was evaluated in rat in vivo. A pathological study was conducted to determine the skin toxicity relationship was investigated by using physicochemical parameters such as a partition coefficient (log *P*).

2. Materials and methods

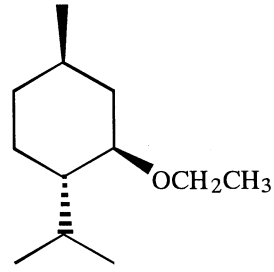
2.1. Materials

Ketoprofen was purchased from Sigma (St. Louis, MO). Capboxyvinyl polymer (HIVISWAKO 105) was generously supplied by Wako (Osaka, Japan). Other chemicals were of reagent grade.

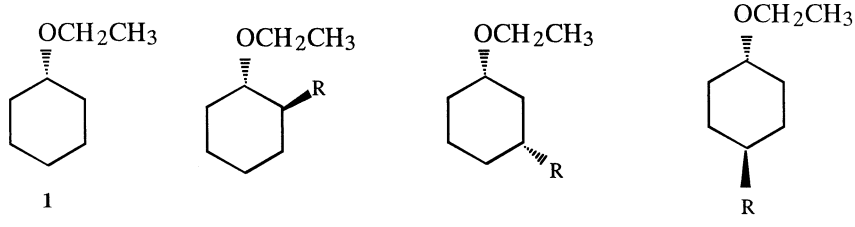
2.2. Synthesis of cyclohexane derivatives

Chemical structures of the synthesized cyclohexanol derivatives are shown in Fig. 1. All the compounds' substituents are equatorial configurations from L-menthol as a lead compound. These compounds were synthesized by five methods. (Group A–E; Group A: compounds 1–3, 8, 14, 15, 19, 23, 25–27, 31, 33; Group B: compounds 20, 22; Group C: compounds 4, 6, 7, 29, 32; Group D: compounds 9, 10, 12, 13, 21; Group E: compounds 5, 11, 16–18, 24, 28, 30, 34, 35). The reaction sequences for the preparation of compounds of group A are outlined in Fig. 2a. The cyclohexanone derivative I was converted into the equatorial alcohol II by reduction with sodium metal in ethanol (Cram and Elhafez, 1952; Vanderwerf and Lemmerman, 1955). The reaction of II by the method described by Meerwein et al. (1950) in dichloromethan gave III in good yields. The compounds of group A–E were prepared by *O*-ethylation in the same manner as equatorial alcohol. The method of preparation of equatorial alcohol of group B–E is described. Group B was synthesized by reduction of cyclohexanone derivatives with sodium borohydoride in methanol in quantitative yields (Fig. 2b). The cyclohexanol derivatives were converted into the 2-substituted compounds by alkylation with alkylharide in the presence of sodium amide (Fig. 2c). Reduction of these compounds in the manner described for the preparation of compounds of group A or B gave good yields of group C. On the other hand, 3-substituted compounds were obtained by 1,4-

addition with alkyl copper regent in ether. These compounds were reduced to group D (Fig. 2d). The group E were synthesized by catalytic reduction of alkylphenol with platinum oxide as a catalyst in a stream of hydrogen (Fig. 2e). But these compounds were axial alcohols. These alcohols were converted into the equatorial alcohol by Jhones oxidation (Epstein et al., 1982) and reduction with sodium metal in ethanol. The purity of each compound was characterized by nuclear magnetic resonance spectroscopy (JEOL PMX 270, Tokyo, Japan), and thin-layer chromatography (silica gel 60, with hexane/dichloromethan = 6/1 as the solvent system). The purity of each compound was over 99%.



O-Ethylmenthol (MET)

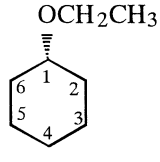


1

	R
2	CH_3
3	CH_2CH_3
4	$CH_2CH_2CH_3$
5	$CH(CH_3)_2$
6	$CH_2(CH_2)_2CH_3$
7	$CH_2(CH_2)_3CH_3$

	R
8	CH_3
9	CH_2CH_3
10	$CH_2CH_2CH_3$
11	$CH(CH_3)_2$
12	$CH_2(CH_2)_2CH_3$
13	$CH_2(CH_2)_3CH_3$

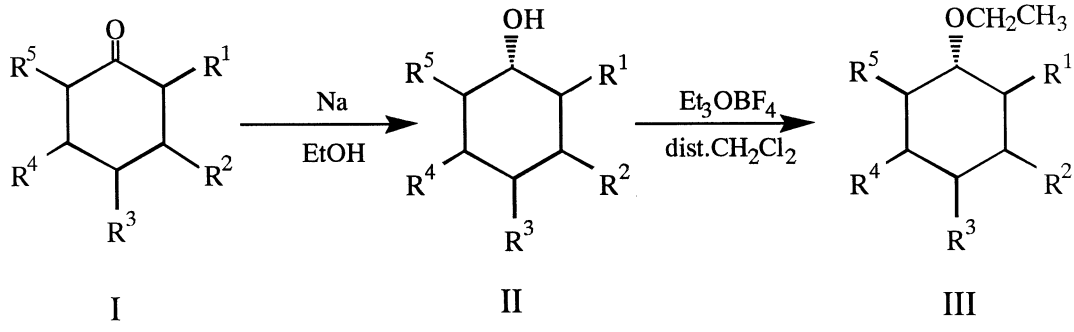
	R
14	CH_3
15	CH_2CH_3
16	$CH_2CH_2CH_3$
17	$CH(CH_3)_2$
18	$CH_2(CH_2)_2CH_3$
19	$CH_2(CH_2)_3CH_3$



	di-CH_3
20	2,2-di CH_3
21	3,3-di CH_3
22	4,4-di CH_3
23	2,3-di CH_3
24	2,4-di CH_3
25	2,5-di CH_3
26	2,6-di CH_3
27	3,4-di CH_3
28	3,5-di CH_3

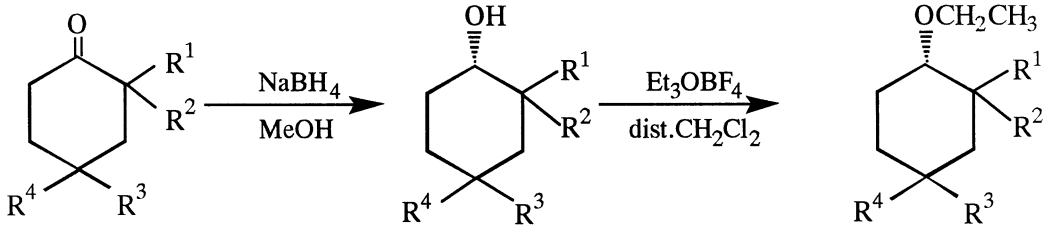
	Others
29	2-CH_3, 4-CH_2CH_3
30	3-CH_3, 4-CH_2CH_3
31	5-CH_3, 2-CH_2CH_3
32	2-CH_3, 4-$CH(CH_3)_2$
33	2-CH_3, 5-$CH(CH_3)_2$
34	3-CH_3, 4-$CH(CH_3)_2$
35	3-CH_3, 5-$CH(CH_3)_2$

Fig. 1. Chemical structure of *O*-ethylmenthol (MET) and synthesized cyclohexanol derivatives.



I II III

(a)



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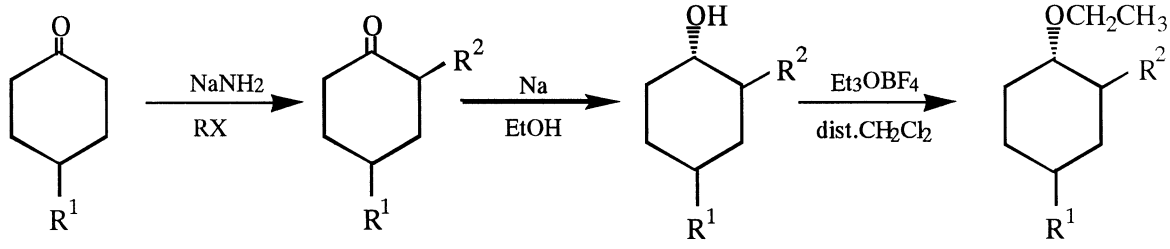

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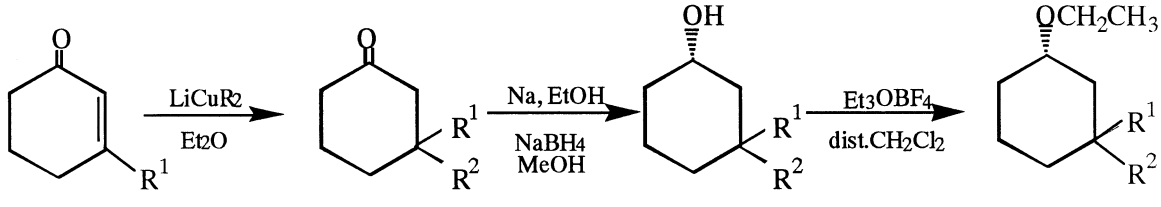

(d)

Fig. 2.

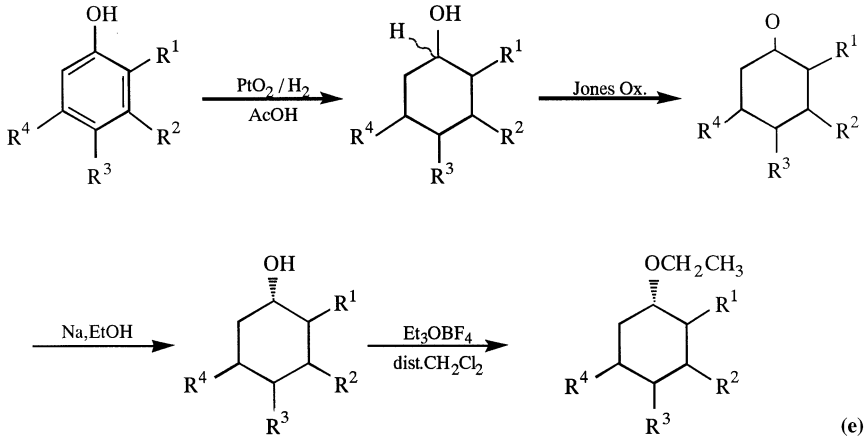

Fig. 2. (a) Reaction sequence for the preparation of compounds 1–3, 8, 14, 15, 19, 23, 25–27, 31, 33 (group A). (b) Reaction sequence for the preparation of compounds 20, 22 (group B). (c) Reaction sequence for the preparation of compounds 4, 6, 7, 29, 32 (group C). (d) Reaction sequence for the preparation of compounds 9, 10, 12, 13, 21 (group D). (e) Reaction sequence for the preparation of compounds 5, 11, 16–18, 24, 28, 30, 34, 35 (group E).

2.3. Preparation of hydrogel

The formulae of the ketoprofen hydrogels used in this study are listed in Table 1. The hydrogels were prepared as follows: carboxyvinyl polymer and triethanolamine were dissolved in distilled water. Separately, ketoprofen and each of the enhancers were dissolved in ethanol. Both solutions were mixed and the resulting hydrogel was stored at room temperature for 24 h under airtight conditions prior to use.

2.4. In vivo percutaneous absorption study

Male Wistar rats weighing 160–180 g were used. After anesthetization with urethane saline solution (25%; 3.0 ml/kg i.p.), the rats were secured on their back, and the hair on the abdominal skin was removed with an electric animal clipper. Glass cells (16 mm inner diameter, 10 mm height) containing the hydrogel under test (1.0 g) were attached to the skin with cyanoacrylate-type adhesives. Blood samples (0.5 ml) were taken via the jugular vein at 1, 2, 4, 6 and 8 h after the administration. Each was centrifuged and the plasma (0.1 ml) was thoroughly mixed with methanol (0.3 ml) containing an appropriate amount of *p*-hydroxybenzoic acid butyl ester as an internal standard. The mixture was again centrifuged for 1 min and the supernatant solution was filtered using a disposable filter unit (Gelman Science Japan, Ekikuro-Disk 3CR). The concentration of ketoprofen in each of the filtrates was then determined using the HPLC method subsequently described.

2.5. Determination of plasma concentration of ketoprofen

The concentration of ketoprofen in the filtrate was analyzed with an HPLC system (Shimadzu, LC-10AS) equipped with a variable wavelength ultraviolet monitor (Shimadzu, SPD-6A). The column was a YMC-Pack A-302 S-5 120A ODS (4.6 × 150 mm; Yamamura Chemical Laboratories). The flow rate was 1.0 ml/min and elution was carried out at room temperature with a mo-

Table 1
Formulae of ketoprofen hydrogels

Ketoprofen	3.0 g
Carboxyvinyl polymer	1.5 g
Triethanolamine	2.0 g
Ethanol	40.0 g
Enhancer	1.0 g
Water	ad 100.0 g

bile phase consisting of 0.057% aqueous phosphoric acid–methanol (35:65). The column effluent was monitored at 254 nm.

2.6. Pathological study

The separated skin was fixed in 10% formalin for at least 24 h before routine processing then cut vertical to the skin surface at the central region in 4 mm sections. Each section was dehydrated using a graded series of ethanol solutions and embedded in paraffin wax. Tissues were divided into small pieces (about 3 μm in thickness) and stained with hematoxylin and eosin. All sections were examined using optiphoto light microscopy.

2.7. Computer program

Physicochemical parameters of cyclohexanol derivatives ($\log P$) were estimated using a computer program 'CAChe' (Oxford Molecular Group, Oxford, UK) with a Power Macintosh 8100/80 computer (Apple Japan, Tokyo, Japan). The $\log P$ was calculated using the atom typing scheme (Ghose et al., 1988). Multiple regression analysis was performed with our own program using an NEC PC-9821 V20 desktop computer (NEC, Tokyo, Japan).

3. Results and discussion

3.1. Promoting activity of synthesized cyclohexanol derivatives

The apparent penetration rate (R_p) was estimated based on the pharmacokinetic model with a constant rate of penetration through the skin after the lag time. The 2-compartment model was applied to the data obtained from the iv administration.

$$C = \frac{R_p}{V_d k_{10}}\left\{1 + \frac{\alpha - k_{10}}{\alpha - \beta}\, e^{-\alpha(t - t_L)} + \frac{k_{10} - \alpha}{\alpha - \beta}\, e^{-\alpha(t - t_L)}\right\} \quad (1)$$

where C is the plasma concentration, R_p is the rate of penetration, t is time, t_L is the lag time, V_d is the distribution volume of the central compartment, k_{10} is the elimination rate constant from the central compartment, and α and β are the hybrid first-order rate constants. The mean values of V_d, k_{10}, α and β, estimated previously (Takayama and Nagai, 1991), were used in this study to determine R_p and t_L values.

As an index to evaluate the promoting activity of each enhancer, an enhancement factor (E_f) was defined as follows:

$$E_f = R_p \text{ (with enhancer)} / R_p \text{ (without enhancer)} \quad (2)$$

As shown in Fig. 3, in the case of mono-substituted compounds (compounds 2–19), the promoting activity of these compounds was the same as or the greater than that of MET except for compound 1, 2, 8 and 14. Compound 5 which has an isopropyl group showed the strongest activity among the compounds that have a substituted group at the C-2 position (compound 2–7). Compounds 11 and 12 were the most effective among that have a substituted group at the C-3 position (compound 8–13). Of the compounds that have a substituted group at C-4 (compounds 14–19), compound 17 which has an isopropyl group showed the strongest activity. These results suggested that the number of carbons in the substitute group is important and there exists an optimum number of carbons in the group.

Of the compounds that have di-substituted groups (compounds 20–35), all were as active or more active than MET. In the series of compounds that have di-methyl groups, compound 20 had the greatest E_f value. In the series of compounds that have methyl and ethyl or methyl and isopropyl groups, compound 29 which has a methyl group at the C-2 position and ethyl group at the C-4 position was the most effective. These results suggested that not only the number of carbons in the substituted group but also the position of the substituted group is important. In the series of compounds that have substituted groups at C-2 and C-4, or C-3 and C-4, the optimum number of carbons in the substitute groups differed. This means that the position of the substituted group is also important.

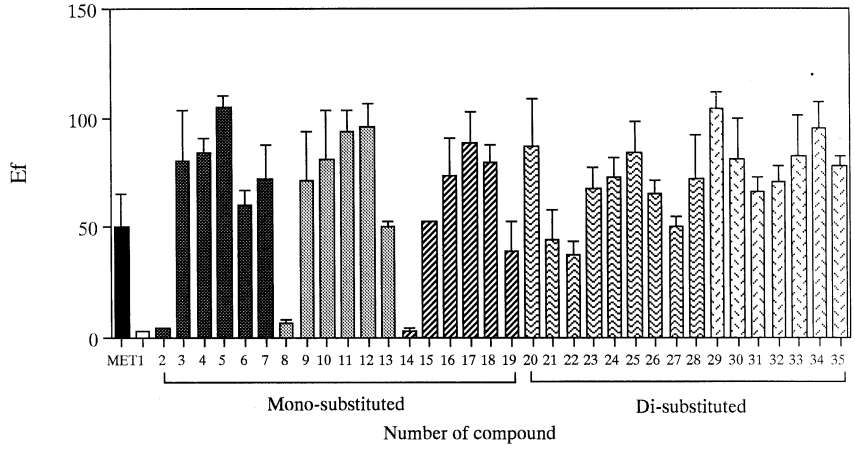

Fig. 3. Enhancement factor (E_f) of *O*-ethylmenthol (MET) and synthesized cyclohexanol derivatives. Each column represents the mean ± S.D. for three determinations.

3.2. Pathological study for cyclohexane derivatives

It is considered important to evaluate skin irritation of enhancer in order to develop safer and more effective promoting agents. At 8 h after the application of hydrogel, the skin was excised and pathologically evaluated. As shown in Table 2, skin irritation was judged by these standards (Lashmar et al., 1989). A total irritation score (TIS) was obtained by summation of the scores of each part and used as an index for skin damage caused by the application of hydrogel.

As shown in Fig. 4, in the case of the compounds that have a mono-substituted group (compound 1–19), the TIS value varied with the promoting activity. However, in the compounds that have a substituted group at C-3 (compound 8–13), the most effective compound (compound 12) did not induce the severest irritation. In the other groups (the compounds that have a substituted group at C-2 or C-4), the promoting activity correlated well with the TIS value. It was considered that compound 12 might be a useful promoting agent. On the other hand when one focused on the irritation of compounds, compound 7 was considered to be preferable because of rater low irritancy and appropriate high promoting efficiency. No damage of skin was evoked by the application of hydrogel without enhancer.

3.3. Structure-activity and structure-irritation relationship

To estimate log *P*, one of the physicochemical properties of molecules, a computer program 'CAChe' was employed. The log *P* was calculated using the atom typing scheme (Ghose et al., 1988). The log *P* values of cyclohexanol derivatives are shown in Table 3. These values calculated CAChe are corresponding to the partition coefficient between octanol/water. The *O*-ethyl cyclohexane (compound 1) was considered to be an anchor for hydrophobic activity and to partition and distribute into the stratum corneum. Hence, it showed a smaller log *P* value than the other com-

Table 2
Judgement indices for skin irritation[a]

Epidermis liquefaction	0–4
Subepidermis edema	0–4
Dermis	
Collagen fiber swelling	0–4
Inflammatory cell infiltration	0–4
Hypodermis	
Collagen fiber swelling	0–4
Inflammatory cell infiltration	0–4
Skin appendages degradation	0–4
Total irritation score (TIS)	0–28

[a] 0, no change; 1, very slight; 2, slight; 3, moderate; 4, marked.

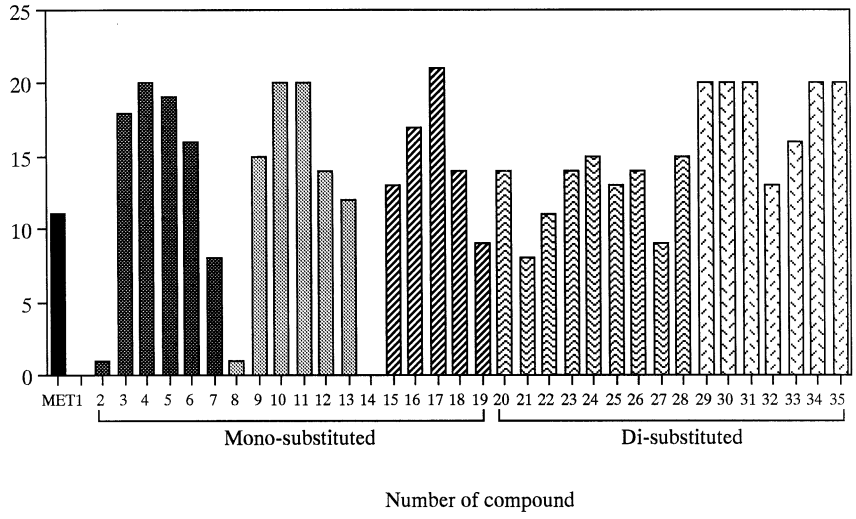

Fig. 4. Total irritation score (TIS) of *O*-ethylmenthol (MET) and synthesized cyclohexane derivatives. Each column represents the mean of three determinations.

pounds. Compound 1 had little effect on the percutaneous absorption of ketoprofen. A more hydrophobic compound was desirable to promote percutaneous absorption of ketoprofen. Although the ring in the chemical structure of enhancer was considered to be critical, it was reported that the size of the ring had little effect on promoting activity and the length of the side chain was more important in the synthesized Azone analogues (Okamoto et al., 1988). Log P values varied between 2.271 (compound 10) and 3.929 (compound 7). Interestingly, the compounds that have the same log P value as MET (compounds 32, 33) showed the greater E_f than MET. From these results, not only the hydrophobicity of compounds but also the position of the substituted group should be taken into consideration. It was observed that effective compounds showed relative severe irritation. The most useful compound among the cyclohexanol derivatives (compound 12) was less lipophilic than MET.

E_f or TIS values were plotted according to log P values of the compounds (Fig. 5). Quantitative relationship was investigated by using multiple regression analysis. An equation for the E_f values was obtained as follows:

$$\log E_f = 6.63(\pm 1.54)\log P - 1.01(\pm 0.26)(\log P)^2 - 8.81(\pm 2.30)$$

$$r = 0.882 \quad s = 0.202 \quad F_0 = 575$$

where r is the multiple regression coefficient, s is the standard deviation of residual and F_0 is the ratio of mean square regression to mean square residual (observed F value). The convex relation between log P and log E_f suggests that there is an optimal lipophilicity of the enhancers for promoting activity. The maximum level of promoting activity was considered to be attained at 3.28 of log P. There also existed the optimum length of tail chain carbons to enhance the permeation of 6-mercaptopurine through the dorsal skin of male guinea pig (Okamoto et al., 1988). In the case of pyrrolidone analogues, a nearly semilogarithmic linear relationship was observed between enhancement potency and the carbon number of the alkyl chain (Yoneto et al., 1995). The structure–activity relationship of chemical enhancers is considered to be very complicated. In this study, ketoprofen was selected as a model permeant. It is considered that the percutaneous absorption of drugs which have different physicochemical characteristics might be shown by the different optimum log P value of the compounds.

An equation for the TIS values was also obtained as a function of log P:

$$\mathrm{Log}(\mathrm{TIS}+1) = 6.11(\pm 1.13)\log P - 0.95(\pm 0.19)(\log P)^2 - 8.52(\pm 1.69)$$

$$r = 0.913 \quad s = 0.149 \quad F_0 = 82.3$$

The parabolic shaped regression curve was also obtained (Fig. 6). This means that the compounds whose log P value was about 3 caused relatively severe irritation. This phenomenon might be influenced by the enhancers in the skin and the clearance of enhancers. Further study should be carried out to clarify the enhancing mechanism of these compounds.

Table 3
Log P of cyclohexane derivatives estimated using the computer program 'CAChe'

No. compound	Log P
MET	3.401
1	1.941
2	2.343
3	2.740
4	3.136
5	3.070
6	3.532
7	3.929
8	2.271
9	2.667
10	3.064
11	2.998
12	3.460
13	3.856
14	2.271
15	2.667
16	3.064
17	2.998
18	3.460
19	3.856
20	2.849
21	2.704
22	2.704
23	2.674
24	2.674
25	2.674
26	2.746
27	2.602
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29	3.070
30	2.998
31	3.070
32	3.401
33	3.401
34	3.328
35	3.328

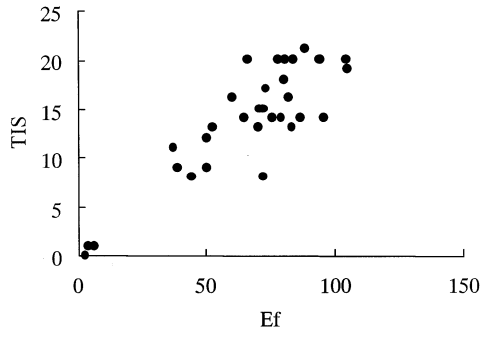

Fig. 5. Relationship between E_f and total irritation score (TIS). Each point represents the mean of three determinations.

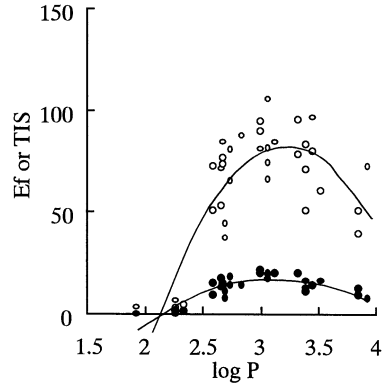

Fig. 6. Relationship between E_f or total irritation score (TIS) and log P. (○): E_f; (●): TIS. Each point represents the mean of three determinations.

4. Conclusion

Synthesized *O*-ethyl cyclohexanol derivatives were evaluated for their promoting activity as a percutaneous absorption enhancer. The compound that has an *iso*-butyl group in the C-3 position (compound 12) showed strong activity to promote percutaneous absorption of ketoprofen in rats and caused relatively little irritation. Other compounds showed strong activity and caused severe irritation.

Acknowledgements

This study was supported by the Ministry of Education, Science, Sports and Culture of Japan. The animal experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Hoshi University which is accredited by the Ministry of Education, Science, Sports and Culture, Japan.

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