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# Pro-drugs for the Oral Delivery of Disodium Cromoglycate[1)]

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Bifunctional pro-drugs of disodium cromoglycate (DSCG), incorporating both lipophilic and hydrophilic pro-moieties, were designed to improve the oral absorption of DSCG, an antiallergic agent used clinically. The synthesized pro-drugs were found to possess the desired properties for an orally active pro-drug. Among the pro-drugs tested, KY-556 (carrying twin ethyl pro-moieties and an L-lysyl pro-moiety) was particularly well absorbed orally and, as expected, displayed good antiallergic activity in the rat passive cutaneous anaphylaxis test.

**Keywords**——disodium cromoglycate; pro-drug; oral absorption; antiallergic activity; physicochemical property

Disodium cromoglycate (DSCG, **1**) has been shown to be useful for the prophylactic treatment of allergic disease states.[2)] DSCG, however, has the disadvantage that it is not orally absorbed and must therefore be administered as an insufflated powder. As a result, the focus of attention for more than a decade has been on the development of more potent, orally active DSCG-like compounds.[3-5)]

The poor oral absorption of DSCG is probably attributable to its low lipophilicity with a strongly acidic character.[2)] The introduction of a lipophilic pro-moiety at each of the acidic carboxy groups can give increased lipophilicity,[6)] but appears to result in loss of water solubility. To overcome the problem of water solubility while maintaining high lipophilicity, we have prepared a novel type of DSCG pro-drug, in which the twin carboxy groups are esterified with lipophilic fragments and, in addition, the hydroxy group is esterified with an amino acid. Such a type of pro-drug may be called a bifunctional pro-drug (**3**) (in contrast to a monofunctional pro-drug (**2**), which incorporates only the lipophilic pro-moieties). Due to the presence of both hydrophilic and lipophilic pro-moieties, bifunctional pro-drugs might be expected to have increased water solubility along with a lipophilic character, and thereby have enhanced oral adsorption.

In this paper, we describe the synthesis and biological properties of some novel bifunctional pro-drugs of DSCG.

## Chemistry

The synthesis of monofunctional and bifunctional pro-drugs was accomplished as shown in Chart 1. The monofunctional pro-drugs (**2**) were prepared by reaction of DSCG with RX (X, halogen). Treatment of **2** with *N*-Boc-amino acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine in dichloromethane yielded intermediates (**3'**) having a protected amino group. The intermediates were treated with HCl–ethanol to remove the protecting group to give the bifunctional pro-drugs (**3**), which were isolated as the hydrochlorides.

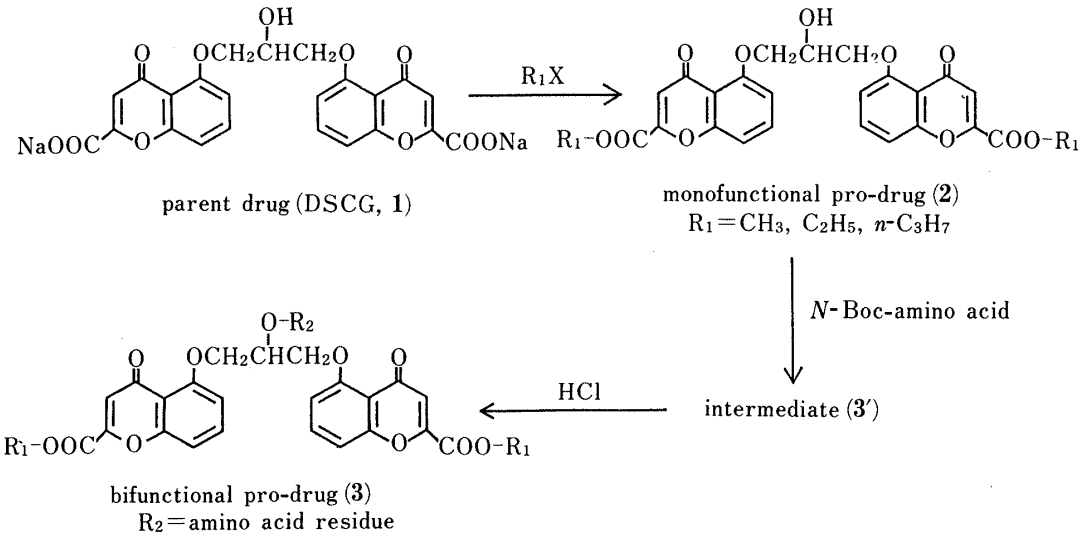



Chart 1

## Results

### Oral Absorption Test in Rabbits

The oral absorption of DSCG pro-drugs was measured by following the urinary excretion of DSCG after oral administration of a dose equivalent to 5 mg/kg of DSCG to rabbits, and the results are shown in Table I.

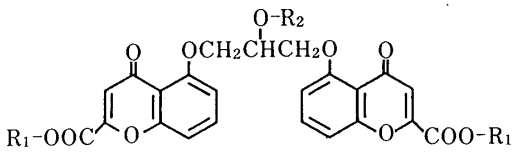
DSCG itself when administered orally showed markedly low urinary recovery. All the monofunctional pro-drugs (**2a**—**c**), incorporating only twin lipophilic pro-moieties ($R_1 = CH_3$, $C_2H_5$ and $n$-$C_3H_7$) at the carboxy groups, failed to give improved oral absorption; the urinary recoveries were less than 6%. In contrast, the bifunctional pro-drugs (**3a**—**h**) in which, in addition to the lipophilic pro-moieties, a hydrophilic pro-moiety ($R_2$ = glycyl, L-alanyl, L-valyl, L-leucyl, L-iso-leucyl, L-prolyl or L-lysyl) was incorporated at the hydroxy group were efficiently absorbed after oral administration, and the urinary recoveries of DSCG were approximately 20—30% of the dose. The pro-drugs were not detected in the urine. Compared with intravenous dosage, these results represent bioavailabilities of approximately 30—40% *via* the oral route. Among the bifunctional pro-drugs tested, **3h** (KY-556: $R_1 = C_2H_5$, $R_2$ = L-lysyl), which exhibited the best oral absorption, was selected for further assessment.

### Physicochemical and Biological Properties of KY-556

**Solubility, Lipophilicity and Hydrolysis of KY-556**——The water solubility and lipophilicity of the parent drug (DSCG), the monofunctional pro-drug (**2b**) and the bifunctional pro-drug (KY-556) are shown in Table II. Compound **2b** was not very soluble in water, while KY-556 was soluble in water to the extent of 35.5 mg/ml. The partition coefficient between 1-octanol and pH 6.5 phosphate buffer was 60.3 for **2b** and 0.166 for KY-556, compared with less than 0.001 for DSCG. KY-556 was hydrolyzed to DSCG in rat intestinal homogenate, with a half-life of 9.45 min.

**Oral Absorption**——The absorption of KY-556 after oral administration of a dose equimolar to 10 mg/kg and 5 mg/kg of DSCG in rats and rabbits, respectively, was compared with that of orally or intravenously administered DSCG (Figs. 1 and 2). DSCG itself was poorly absorbed orally; the peak levels in plasma were 0.04 $\mu$g/ml in rats and below the detection limit (0.025 $\mu$g/ml) in rabbits. In contrast, KY-556 was well absorbed orally, and delivered as DSCG to the peripheral circulation, resulting in peak levels of 0.24 and 0.97 $\mu$g/ml in rats and rabbits, respectively. The bioavailability of KY-556 was calculated to be

TABLE I. Physicochemical and Biological Properties of DSCG Pro-drugs



| Compd. No. | $R_1$ | $R_2$ | mp (°C) | Formula[a] | Urinary recovery (%)[b] |
|---|---|---|---|---|---|
| **1** (DSCG)[c] | Na | H | — | — | 69.0 (i.v.)<br>3.5 (*p.o.*) |
| **2a** | $CH_3$ | H | 205 | $C_{25}H_{20}O_{11}$ | 1.5 |
| **2b**[d] | $C_2H_5$ | H | 186 | $C_{27}H_{24}O_{11}$ | 5.0 |
| **2c** | $n$-$C_3H_7$ | H | 170 | $C_{28}H_{26}O_{11}$ | 5.9 |
| **3a** | $CH_3$ | L-Alanyl·HCl | Amorphous | $C_{28}H_{25}NO_{12}\cdot HCl$ | 17.7 |
| **3b** | $C_2H_5$ | Glycyl·HCl | 202—205 | $C_{29}H_{27}NO_{12}\cdot HCl$ | 24.3 |
| **3c** | $C_2H_5$ | L-Alanyl·HCl | 185.5—187.5 | $C_{30}H_{29}NO_{12}\cdot HCl$ | 20.2 |
| **3d** | $C_2H_5$ | L-Valyl·HCl | 185—189 | $C_{32}H_{33}NO_{12}\cdot HCl$ | 19.9 |
| **3e** | $C_2H_5$ | L-Leucyl·HCl | 172—174 | $C_{33}H_{35}NO_{12}\cdot HCl$ | 22.8 |
| **3f** | $C_2H_5$ | L-iso-Leucyl·HCl | 178—183 | $C_{33}H_{35}NO_{12}\cdot HCl$ | 11.8 |
| **3g** | $C_2H_5$ | L-Prolyl·HCl | 110 | $C_{32}H_{31}NO_{12}\cdot HCl$ | 24.6 |
| **3h** (KY-556) | $C_2H_5$ | L-Lysyl·2HCl | 225—228 | $C_{33}H_{36}N_2O_{12}\cdot 2HCl$ | 29.6 |

*a*) All compounds were analyzed for C, H and N; the analytical results were within ±0.4% of the calculated values. *b*) During 6 h after oral administration of a dose equivalent to 5 mg/kg of DSCG in rabbits ($n=3$). *c*) Commerical product. *d*) H. Cairns, C. Fitzmaurice, D. Hunter, P. B. Johnson, J. King, T. B. Lee, G. H. Lord, R. Minshull, and J. S. G. Cox, *J. Med. Chem.*, **15**, 583 (1972).

TABLE II. Solubility, Partition Coefficient and Hydrolyzability of DSCG Pro-drugs

| Compd. | Water solubility (mg/ml) | Partition coefficient[a] | Hydrolysis[b] $t_{1/2}$ (min) |
|---|---|---|---|
| **1** (DSCG) | 195.3 | <0.001 | |
| **2b** | 0.0052 | 60.3 | |
| **3h** (KY-556) | 35.5 | 0.166 | 9.45 |

*a*) pH 6.5 buffer–1-octanol. *b*) In 10% rat intestine homogenate at 37 °C.

15.6% in rats and 43.7% in rabbits from the ratio of the areas under the plasma level–time curves for the oral dose of KY-556 and the intravenous dose of DSCG.

**Rat Passive Cutaneous Anaphylaxis Test**——KY-556 was evaluated for antiallergic activity in the rat passive cutaneous anaphylaxis (PCA) test. The time course of inhibitory action of KY-556 on 48 h PCA when administered orally is shown in Fig. 3. The inhibitory activity was most potent at 30 min pretreatment. The inhibitory activity of KY-556 after doses of 5, 10 and 20 mg/kg in 30 min pretreatment is shown in Fig. 4 and compared with that of Tranilast® at 20 mg/kg. KY-556 displayed good antiallergic activity in a dose-dependent manner, and its activity was better than that of Tranilast®.

## Discussion

It is well known that efficient absorption of a pro-drug from the gastrointestinal tract requires the function of three processes; (1) dissolution in the gastrointestinal fluids, (2)

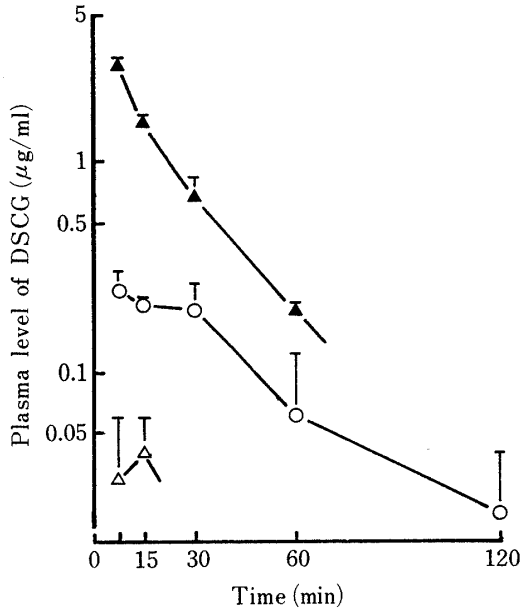



Fig. 1. Plasma Levels of DSCG after Oral Administration of KY-556 (Equivalent to 10 mg/kg of DSCG), and Oral and Intravenous Administration of DSCG (10 mg/kg) in Rats

—○—, KY-556 *p.o.*; —▲—, DSCG i.v.; —△—, DSCG *p.o.*

Each point represents the mean ± S.E. for three rats.

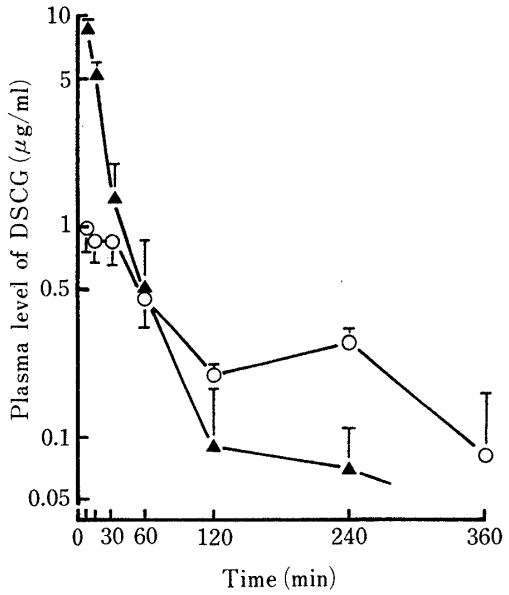



Fig. 2. Plasma Levels of DSCG after Oral Administration of KY-556 (Equivalent to 5 mg/kg of DSCG), and Oral and Intravenous Administration of DSCG (5 mg/kg) in Rabbits

—○—, KY-556 *p.o.*; —▲—, DSCG i.v.

Each point represents the mean ± S.E. for three rabbits.

In the case of DSCG oral administration, the plasma level was lower than the detection limit (0.025 μg/ml) at every point.

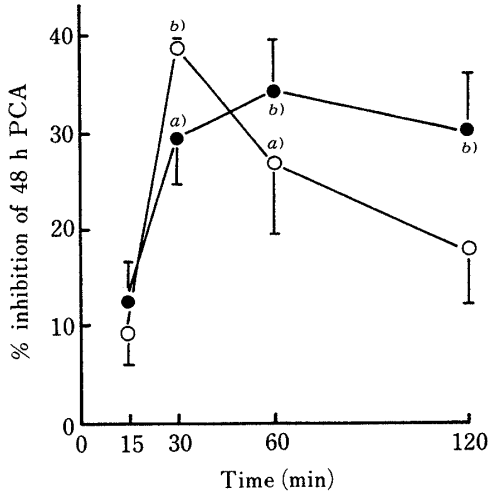



Fig. 3. Time Course of Inhibitory Actions on 48 h Homologous PCA after Oral Administration of KY-556 and Tranilast® at Doses of 20 mg/kg in Rats

—○—, KY-556 *p.o.*; —●—, Tranilast® *p.o.*

Each point represents the mean ± S.E. for five rats.

*a*, *b*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively (Student's *t* test).

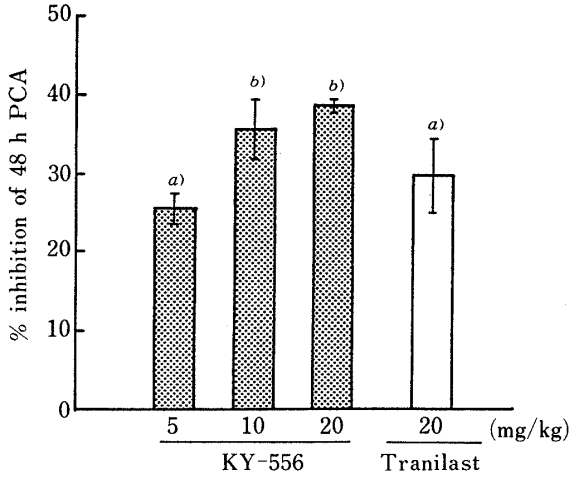


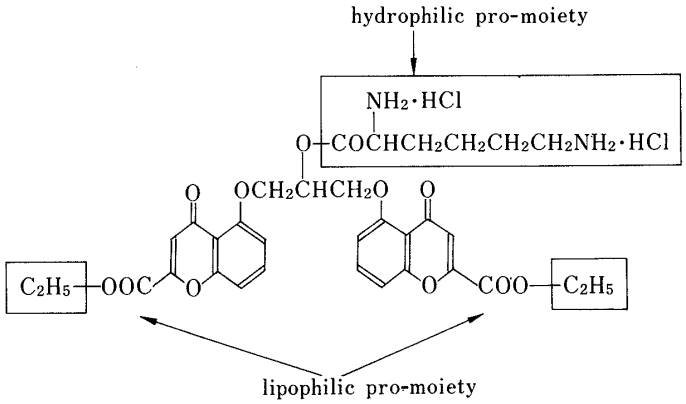

Fig. 4. Inhibitory Effects of KY-556 and Tranilast® on 48 h Homologous PCA in Rats

KY-556 and Tranilast® were administered orally 30 min before challenge with the antigen (EWA).

Each column represents the mean ± S.E. for five rats.

*a*, *b*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively (Student's *t* test).

transport across the gastrointestinal membrane, and (3) reversion to the parent drug at the right time and place.[7] The physicochemical factors governing processes 1, 2 and 3 appear to be mainly water-solubility, lipophilicity and hydrolysis rate, respectively.




KY-556

Chart 2

In this study, DSCG was poorly absorbed orally due to its very low lipophilicity. The monofunctional pro-drugs (**2a**, **b**), despite the introduction of twin lipophilic pro-moieties, did not show any increase in oral absorption (Table I). This result may be ascribed to their markedly decreased water solubility occurring in parallel with increased lipophilicity.

In contrast, the bifunctional pro-drugs incorporating twin lipophilic pro-moieties and a hydrophilic pro-moiety exhibited substantial improvements in oral absorption over DSCG (Table 1). In particular, KY-556 carrying an L-lysyl and twin ethyl moieties (Chart 2) was found to have physicochemical properties (solubility, lipophilicity and hydrolyzability) favorable for an orally active pro-drug (Table II) and, as expected, showed markedly improved absorption (Figs. 1 and 2). Also, as we would expect from the good oral absorption, KY-556 displayed potent antiallergic activity in the rat PCA test (Figs. 3 and 4).

As to the modes of oral absorption and action of KY-556, it is reasonable to assume that during absorption of the pro-drug from the gastrointestinal tract both L-lysine and ethanol are split off enzymatically with the formation of DSCG, which passes into the systemic circulation through the portal vein and the liver, and then exhibits antiallergic activity.

In conclusion, KY-556 appears to be promising as a candidate pro-drug for the oral delivery of DSCG.

## Experimental

Melting points were determined with a Yamato MR-21 apparatus and are uncorrected. The nuclear magnetic resonance (NMR) spectra were obtained on a Hitachi R-600 spectrometer. Infrared (IR) spectra were obtained in KBr on a Shimadzu IR-400 spectrometer. Elemental analysis was performed by the microanalytical group of Kyoto University.

**1,3-Bis(2-methoxycarbonylchromon-5-yloxy)propan-2-ol (2a)**——Methyl iodide (1.22 ml) was added to a stirred suspension of DSCG (2 g) in *N*,*N*-dimethylformamide (DMF) (80 ml) at 10 °C. After being stirred for 4 h at 40°C, the reaction mixture was poured into $CHCl_3$ (200 ml) and the insoluble material was filtered off. The $CHCl_3$ layer was washed with water, 5% $NaHCO_3$ solution and brine, and then dried over $Na_2SO_4$. The organic solution was filtered and evaporated *in vacuo* to give 1.44 g of **2a**. IR (KBr): 3430, 1735, 1650 $cm^{-1}$. NMR ($CDCl_3$) $\delta$: 1.5—2.2 (1H, br), 3.98 (6H, s), 4.1—4.8 (4H, m), 6.7—7.4 (6H, m), 7.58 (2H, t, $J$=9 Hz). *Anal.* Calcd for $C_{25}H_{20}O_{11}$: C, 60.48; H, 4.06. Found: C, 60.44; H, 4.27.

**1,3-Bis(2-ethoxycarbonylchromon-5-yloxy)propan-2-ol (2b)**——Ethyl iodide (125 g) was added dropwise during 2 h to a stirred suspension of DSCG in *N*,*N*-dimethylacetamide (DMA) (3 l) at 64—70 °C. The mixture was stirred for 5 h at the same temperature, then the insoluble material was filtered off. The organic filtrate was allowed to stand overnight at 4—6 °C. The resulting crystalline precipitate was collected, washed with water and AcOEt, and dried *in vacuo* to give 136 g of **2b**. IR (KBr): 1650 $cm^{-1}$. NMR ($CDCl_3$) $\delta$: 1.41 (6H, t, $J$=7 Hz), 4.42 (4H, q, $J$=7 Hz), 4.45 (6H, br), 6.91 (2H, s), 6.8—7.3 (4H, m), 7.57 (2H, t, $J$=9 Hz). *Anal.* Calcd for $C_{27}H_{24}O_{11}$: C, 61.83; H, 4.61. Found:

C, 61.64; H, 4.60.

**2-[*N,N'*-Di(*tert*-butoxycarbonyl)-L-lysyloxy]-1,3-bis(2-ethoxycarbonylchromon-5-yloxy)propane (3'h)**——Dimethylaminopyridine (0.38 g) was added to a stirred suspension of **2b** (3.2 g) in $CH_2Cl_2$ (50 ml), and then a solution of di-Boc-L-lysine (3.2 g) in $CH_2Cl_2$ (20 ml) and DCC (1.9 g) were added. The mixture was stirred for 3 h, the precipitated dicyclohexylurea (DCU) was filtered off, and the filtrate was washed with 5% citric acid solution, 5% $NaHCO_3$ solution and brine, then dried over $Na_2SO_4$. The solvent was evaporated off *in vacuo* to give 4.7 g of **3'h**. IR (KBr): 1740, 1710, 1690, 1655 $cm^{-1}$.

**2-(L-Lysyloxy)-1,3-bis(2-ethoxycarbonylchromon-5-yloxy)propane Dihydrochloride (KY-556, 3h)**——A 5 N HCl–ethanol solution (54 ml) was added to a solution of **3'h** (23 g) in $CHCl_3$ (110 ml) at 5°C. The mixture was stirred for 50 min, then isopropyl ether was added. The precipitated solid was collected and recrystallized from $CHCl_3$–ethanol to give 16 g of **3h**. IR (KBr): 1740 $cm^{-1}$. NMR (DMSO)$\delta$: 1.34 (6H, t, $J$=7 Hz), 1.4—2.2 (6H, m), 2.3—3.0 (2H, m), 3.7—4.2 (1H, m), 4.36 (4H, q, $J$=7 Hz), 4.3—4.9 (4H, m), 5.4—5.9 (1H, m), 6.72, 6.74 (2H, s), 7.12, 7.19 (4H, d, $J$=9 Hz), 7.74 (2H, t, $J$=9 Hz), 7.6—9.0 (6H, br). *Anal.* Calcd for $C_{33}H_{36}N_2O_{12}\cdot 2HCl\cdot 2H_2O$: C, 52.04; H, 5.29; N, 3.68. Found: C, 51.85; H, 5.29; N, 3.75.

**Water Solubility**——DSCG, **2b** or KY-556 (500 mg) was added to water (10 ml) and shaken for 2 h at 25°C. After filtration, the concentration of the compound was measured by the high-performance liquid chromatographic (HPLC) method.

**Partition Coefficient**——DSCG, **2b** or KY-556 (10 mg) was added to 50 ml of a 1/15 M phosphate buffer (pH 6.5). The aqueous solution (DSCG) or the suspension (**2b** or KY-556) was shaken vigorously with 50 ml of 1-octanol at 25°C. When equilibrium had been achieved (1 h), the aqueous layer was separated by centrifugation, and the concentration of the compound was measured by the HPLC method.

***In Vitro* Hydrolysis**——The small intestine was obtained from a freshly killed rat. It was homogenized at 10% w/v in ice-cold saline, using an ultra disperser (LK-21, Yamato). KY-556 was dissolved in saline at a concentration equivalent to 500 $\mu$g/ml of DSCG. The solution (0.2 ml) was rapidly added to the intestinal homogenate (1.8 ml) to give a reaction mixture containing KY-556 at a final concentration of 50 $\mu$g/ml in 10% w/v homogenate. This was incubated at 37°C and sampled at 0, 2, 5, 10 and 30 min after mixing. Samples of 0.2 ml were added to 0.4 ml of $CH_3CN$ containing $10^{-4}$ M diisopropylfluorophosphate (DFP; esterase inhibitor) and shaken vigorously. After centrifugation, the supernatant was assayed by the HPLC method.

**Absorption Studies**——Urinary Recovery in Rabbits: Male rabbits weighing 3—4 kg were starved overnight. DSCG was administered to the test animals orally or intravenously as an aqueous solution, at a dose of 5 mg/kg. Compounds **2a—c** and **3a—h**, prepared as aqueous suspensions and aqueous solutions, respectively, were administered orally to the animals at the dose equivalent to 5 mg/kg of DSCG. The DSCG concentrations in the urine samples, collected in a metabolic cage during 6 h after administration, were measured by the HPLC method.

Plasma Levels in Rabbits and Rats: Male Wistar strain rats weighing 180—220 g and male rabbits weighing 3—4 kg were used. Before the experiment, the animals were starved overnight but were allowed to drink water. DSCG, prepared as an aqueous solution, was administered orally or intravenously at a dose of 5 mg/kg to rabbits and at a dose of 10 mg/kg to rats. KY-556 was administered orally to the test animals as an aqueous solution at the same dose (equivalent to DSCG). Plasma samples were drawn at 7.5, 15, 30, 60, 120 and 240 min after administration of the compounds. The concentrations of DSCG in the plasma were determined by the HPLC method.

**Analytical Method**——DSCG and KY-556 in the samples were measured by HPLC under the following conditions: a TRI ROTAR liquid chromatograph equipped with a UVIDEC-100-II UV-detector (Japan Spectroscopic Co., Ltd.) and a $\mu$-Bondapak $NH_2$ column for DSCG or a $\mu$-Bondapak $C_{18}$ column for KY-556 was used. The mobile phase consisted of 0.05 M $KH_2PO_4$ (pH 3.0)–$CH_3CN$ (70 : 30) to detect DSCG and 0.05 M $KH_2PO_4$ (pH 4.5)–$CH_3CN$ (55 : 45) to detect KY-556, and the flow rates were 3.0 ml/min. Pretreatment of plasma and urine samples was carried out according to the procedure reported by Kobayashi and Machida[8] with some modification.

**Rat Passive Cutaneous Anaphylaxis**——Male Wistar strain rats, weighing 160—175 g, and female Wistar strain rats weighing 180—200 g were used for the PCA test and the preparation of antiserum, respectively. Anti-egg white albumin (anti-EWA) serum was prepared according to Stotland and Shore.[9] Rats were sensitized by intradermal injection of 0.05 ml of anti-EWA serum, diluted 10-fold with saline, into the dorsal skin. After 48 h, the rats were challenged with an intravenous injection of 0.5 ml of 1% Evans Blue solution containing 5 mg of EWA. The drug was administered orally at 15, 30, 60 or 120 min before challenge with the antigen. The animals were exsanguinated 30 min after challenge, and the skin was removed to measure the PCA blueing lesion. The amount of dye was measured colorimetrically after extraction by the method of Katayama *et al.*[10]

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## References and Notes

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