[Chem. Pharm. Bull. 36(6)2128—2134(1988)]

KY-109, a New Bifunctional Prodrug of Cephalosporin. II.[1] Mechanism of Oral Absorption

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(Received September 14, 1987)

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl (6*R*,7*R*)-7-[(*R*)-2-[(*S*)-alanyloxy]-2-phenylacetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride (KY-109) is a bifunctional prodrug designed to improve the oral absorption of the parent drug (KY-087), which is a cephalosporin with a broad spectrum of antibacterial activity.

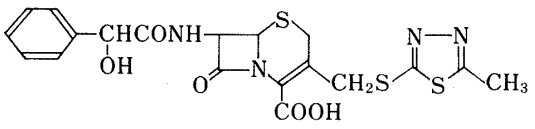
The mechanism of oral absorption of KY-109 was investigated in rats. An aqueous solution of KY-087 and a suspension of KY-106, which is esterified with a (5-methyl-2-oxo-1,3-dioxol)methyl group at the C-4 carboxy group of KY-087, were poorly absorbed orally in rats. However, a 50% propylene glycol solution of KY-106 and an aqueous solution of KY-109 were well absorbed. KY-109 was rapidly hydrolyzed to KY-106 in the small intestinal contents, though it was hardly hydrolyzed in the stomach contents. Further, KY-109 was hydrolyzed to KY-087 *via* KY-106 in the gastrointestinal homogenate.

From these results, the mechanism of the oral absorption of KY-109 can be speculated to be as follows. KY-109 is transferred into the small intestinal lumen without hydrolysis in the stomach, and is then hydrolyzed to KY-106 with the release of the alanyl residue. The resulting KY-106 is then transferred into the mucosal membrane, and hydrolyzed enzymatically with the formation of KY-087 along with acetoin. The resulting KY-087 is then transferred into the blood stream.

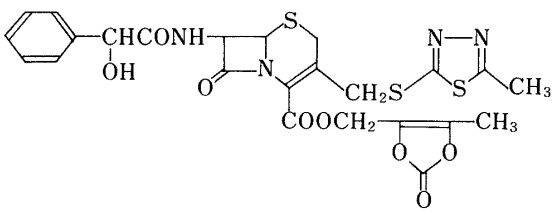
Keywords——prodrug; cephalosporin; oral absorption; hydrolysis; absorption mechanism

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl (6*R*, 7*R*)-7-[(*R*)-2-[(*S*)-alanyloxy]-2-phenylacetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride (KY-109) is an orally active prodrug of (6*R*, 7*R*)-7-[(*R*)-2-hydroxy-2-phenylacetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl]-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylic acid (KY-087), which is a cephalosporin with a broad spectrum of antibacterial activity.[1] For this prodrug, in order to increase its oral absorption, the lipophilicity has been improved over that of KY-087 (the parent drug) by means of esterification of the C-4 carboxy group with a (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl (DOX) group and, moreover, the reduction of its aqueous solubility caused by the introduction of DOX has been depressed through esterification of the side-chain α-hydroxy group with L-alanine. Such a prodrug, which possesses both lipophilic and hydrophilic promoieties, has been called a bifunctional prodrug, in contrast to monofunctional prodrugs (KY-106 or KY-153) which incorporate either a lipophilic or a hydrophilic promoiety (Chart 1).

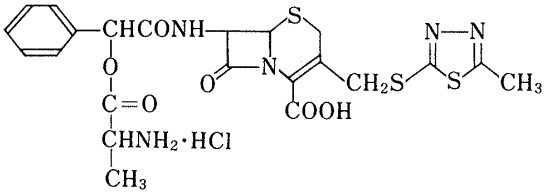
It has been previously reported[1] that KY-109 possesses properties of lipophilicity, aqueous solubility and hydrolysis in biological fluids that should make it an orally active prodrug; indeed, KY-109 when administered orally to rats, was well absorbed, giving high blood levels of the parent drug.



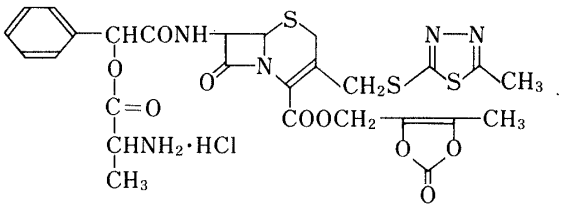
parent drug (KY-087)



monofunctional prodrug (KY-106)



monofunctional prodrug (KY-153)



bifunctional prodrug (KY-109)

Chart 1

In this paper, we describe the results of a study of the gastrointestinal absorption of KY-109.

Materials and Methods

Chemicals——KY-109, KY-106, KY-087 and KY-153 were prepared in the Research Laboratories of Kyoto Pharmaceutical Industries, Ltd.; their activities were 674, 807, 935 and 850 μg/mg, respectively.

Animals——Male Sprague-Dawley rats, aged 7 weeks, were used. Before the experiment, rats were fasted overnight but water was freely available.

Absorption Studies——Test solutions of KY-109, KY-153 and KY-087 were prepared in 0.5% methylcellulose (MC) at a concentration of 2 mg/ml. Since KY-106 has a very low solubility in water, the test solution was prepared by dissolving it in propylene glycol at about 50 °C, and then diluting with an equal volume of warm saline to produce a final drug concentration of 2 mg/ml. In addition, a suspension of KY-106 was prepared in 0.5% MC at 2 mg/ml. The drugs were administered orally at a dose equivalent to 20 mg/kg of KY-087 to rats.

Blood samples were collected from the jugular vein at specified intervals after administration. The concentrations of KY-087 in the serum were determined by the disc-plate diffusion method using *Bacillus subtilis* ATCC 6633 as the test organism and sodium citrate agar (sodium citrate 1.0%, polypeptone 0.5%, beef extract 0.3%, agar 1.5%) as the test medium. Standard solutions were prepared with serum from control rats.

***In Situ* Absorption Studies**——The method used for the *in situ* absorption studies was based on that of Noguchi *et al.*[2] with suitable modification. Rats were injected intraperitoneally with 25 mg/kg of sodium pentobarbital to induce anesthesia. The stomach, the small intestine, the cecum and the colon were exposed by a midline abdominal incision and the bile duct was ligated. Loops were formed by ligating each end of the stomach, the cecum, the colon (10 cm) and the upper part (10 cm), the middle part (10 cm) and the lower part (10 cm) of the small intestine. One milliliter of the drug solution (2 mg/ml) was injected into each loop. The gastrointestinal tract was then replaced in the abdominal cavity and the incision was closed. After injection, 0.6-ml blood samples were collected from the jugular vein at specified intervals.

The concentrations of KY-087 in the serum were bioassayed according to the method described for the absorption studies.

Stability of KY-109 in the Gastrointestinal Contents and Gastrointestinal Tissue Homogenate——Rats were sacrificed by exsanguination, and the gastrointestinal tracts were removed and divided into four areas, *i.e.*, the stomach, small intestine, cecum and colon. The contents of the stomach, small intestine, cecum and colon were washed out with 2.5, 6.0, 4.0 and 2.5 ml of saline, respectively; the washings from three rats were combined and mixed. Specimens were prepared by centrifugation at 10000 rpm for 5 min at 4 °C. The pH values of specimens were 3.3 for the stomach and 6.8—7.2 for the small intestine, cecum and colon. The gastrointestinal tissues from three rats were combined and homogenized with an Ultra Disperser (LK-21, Yamato) after the addition of 2 volumes of saline, and centrifuged at 10000 rpm for 15 min at 4 °C. The supernatant pH values were 6.4—6.5. To 4.5 ml of each specimen was added 0.5 ml (1 mg/ml) of KY-109 aqueous solution; the mixtures were incubated at 37 °C. Sampling was carried out at specified intervals after mixing. The sample was added to 2 volumes of acetonitrile and shaken.

After centrifugation at 10000 rpm for 15 min, the concentrations of KY-109, KY-106, KY-153 and KY-087 in the supernatant were measured by high-performance liquid chromatography (HPLC; Waters ALC/G2C Compact Type, model 441 detector, 254 nm filter, model 45 pump, model WISP 710B injector, and reverse-phase Radialpak μ-Bondapak C_{18} column). Elution was carried out with 0.05 M $NaHPO_4$–CH_3CN (81 : 19 for KY-087 and KY-153, and 62 : 38 for KY-109 and KY-106) at a flow rate of 3.0 ml/min.

Active Metabolites in Portal Blood——KY-109 solution (20 mg/ml) prepared with 0.5 w/v% MC was administered orally to rats at a dose of 100 mg/kg (5 ml/kg). Portal blood was taken from the portal vein with a heparin-treated syringe through a midline abdominal incision under anesthesia with ether at specified intervals after administration. Each blood sample was placed in a tube pretreated with two drops of 3×10^{-2} M diisopropylfluorophosphate (esterase inhibitor; Sigma) to prevent the hydrolysis of ester prodrugs in the blood and centrifuged at 3000 rpm for 5 min. Then 0.4 ml of acetonitrile was added to 0.2 ml of the supernatant. This mixture was centrifuged at 10000 rpm for 5 min after shaking. Twenty microliters of the supernatant was spotted onto a silica-gel thin-layer chromatography plate (DC-Plastikfolien Kieselgel 60, Merck) and the chromatogram was developed with chloroform–ethanol–formic acid (10 : 2 : 1). The plate was air-dried after development and sprayed with esterase solution extracted from malt. Antibacterial activity was detected by placing the plate on an agar plate seeded with *Bacillus subtilis* ATCC 6633 for 10 min followed by incubation for 18 h at 37 °C. The spots detected on the bioautogram were identified by comparing their *Rf* values with those of authentic samples of KY-109, KY-106, KY-153 and KY-087.

Results

Oral Absorption in Rats

The absorption of KY-109 (aqueous solution) in rats after oral administration of a dose equivalent to 20 mg/kg of KY-087 was compared with that of orally administered KY-087 (aqueous solution), KY-153 (aqueous solution), and KY-106 (suspension of 0.5% MC or a solution of 50 v/v% propylene glycol) (Fig. 1). Aqueous solutions of KY-087 and KY-153 and the suspension of KY-106, when administered orally, were poorly absorbed, the peak levels of KY-087 in serum being 1.02 μg/ml at 30 min, 0.71 μg/ml at 30 min and 0.25 μg/ml at 2 h, respectively. In contrast, the aqueous solution of KY-109 and the 50% propylene glycol

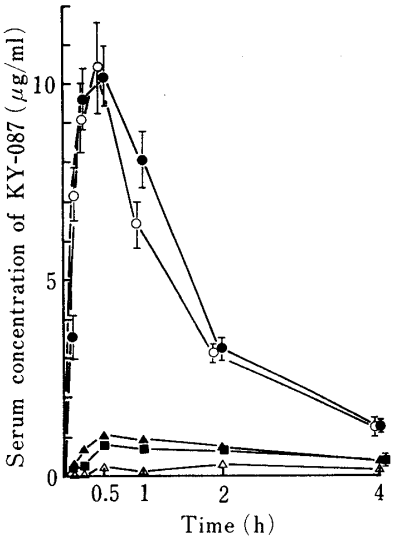


Fig. 1. Serum Levels of KY-087 after Oral Administration of KY-109, KY-106, KY-153 and KY-087 in Rats

Dose: equivalent to 20 mg/kg of KY-087; —●—, KY-109 aqueous solution; —○—, KY-106 50 v/v% propylene glycol solution; —△—, KY-106 0.5% MC suspension; —■—, KY-153 aqueous solution; —▲—, KY-087 aqueous solution. Each point represents the average of six or ten experiments; the vertical bar represents the standard error of the mean.

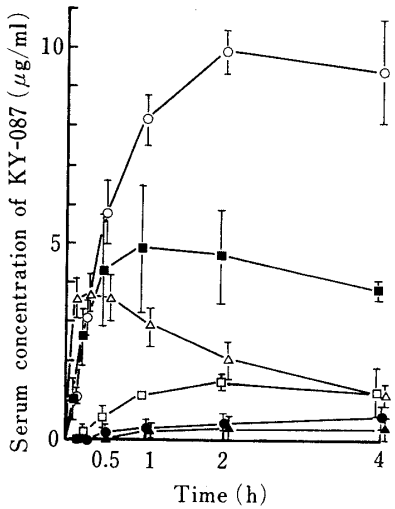


Fig. 2. Serum Levels of KY-087 after Injection of KY-109 into the Gastrointestinal Loop of Rats

Dose: equivalent to 2 mg/loop; —●—, stomach; —○—, small intestine (upper); —■—, small intestine (middle); —□—, small intestine (lower); —▲—, cecum; —△—, large intestine. Each point represents the average of three experiments; the vertical bar represents the standard error of the mean.

solution of KY-106 were well absorbed, the peak levels of KY-087 being 10.17 μg/ml at 30 min and 10.40 μg/ml at 30 min, respectively.

Absorption Site

The serum levels of KY-087 after administration of KY-109 into the gastrointestinal loops of rats are shown in Fig. 2. KY-109 was absorbed mainly from the upper small intestine and partly from the middle intestine, and a little was absorbed from the lower intestine. In addition, a certain amount was absorbed from the colon, whereas the absorption from the stomach and cecum was poor.

Stability of KY-109 in the Gastrointestinal Contents and Gastrointestinal Tissue Homogenate

The stability of KY-109 in the rat gastrointestinal contents and gastrointestinal tissue homogenate are shown in Figs. 3 and 4. KY-109 was stable in the stomach contents; formation of KY-087, KY-106 and KY-153 was only slightly observed. The amounts of residual KY-109 in the stomach contents were 89.3% and 77.1% after 2 and 6 h of incubation, respectively. On the other hand, KY-109 was rapidly hydrolyzed in the contents of the small intestine, cecum and colon, and could not be detected after 30 min. KY-106 was mainly produced, its rates of formation being 50.5%, 62.9% and 77.2% in the contents of the small intestine, cecum and colon, respectively. KY-109 was rapidly hydrolyzed in all parts of the gastrointestinal homogenate and was undetectable 30 min after the start of incubation. The level of KY-106 produced was at a maximum 5 min after the start of incubation in the homogenates of the stomach, small intestine and cecum, the rates being 37.6%, 57.9% and 58.9%, respectively. In the colon homogenate, the level of KY-106 produced was at a maximum after 10 min, being 39.8%. Moreover, the resulting KY-106 in each homogenate was rapidly hydrolyzed, and the amounts of residual KY-106 after 30 min of incubation were 5.9%, 24.6%, 12.3% and 0% in the homogenates of the stomach, small intestine, cecum and colon, respectively. The rates of formation of KY-087 in each homogenate were remarkably higher than those in the gastrointestinal contents. These values were 69.4%, 71.2%, 73.0% and

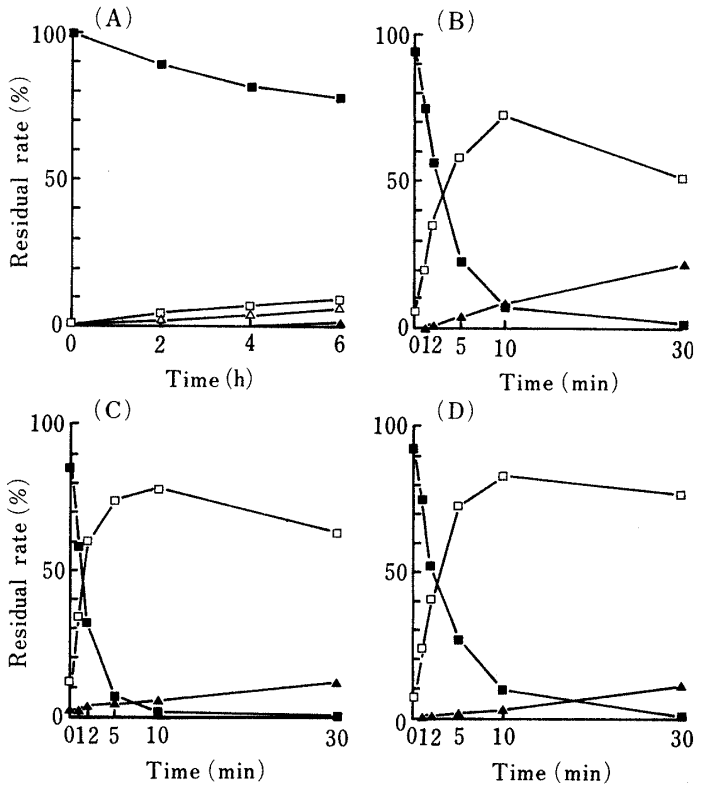


Fig. 3. Stability of KY-109 in Gastrointestinal Contents of Rats

A, stomach; B, small intestine; C, cecum; D, large intestine. —■—, KY-109; —□—, KY-106; —△—, KY-153; —▲—, KY-087. The initial concentration of KY-109 was 100 μg/ml.

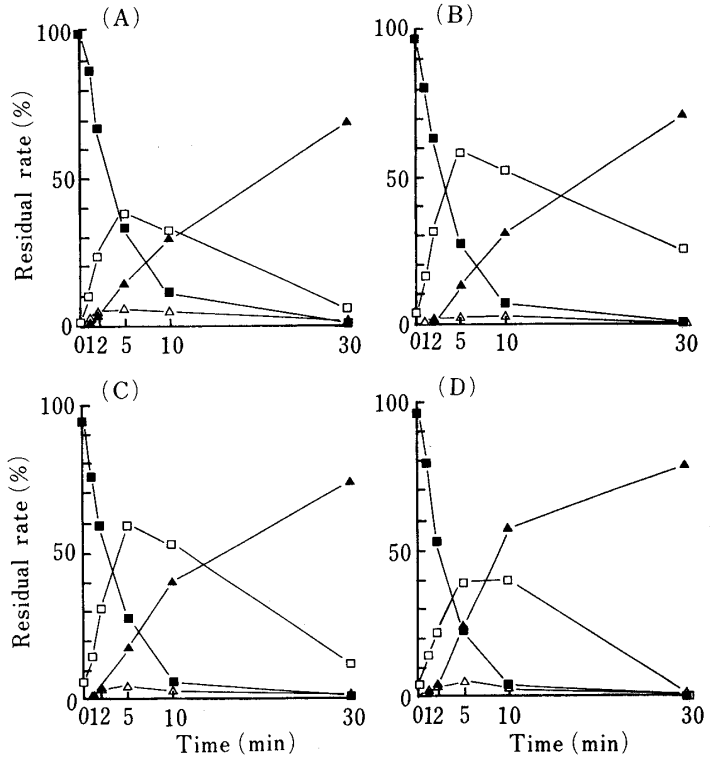


Fig. 4. Stability of KY-109 in Gastrointestinal Homogenates of Rats

A, stomach; B, small intestine; C, cecum; D, large intestine. —■—, KY-109; —□—, KY-106; —△—, KY-153; —▲—, KY-087. The initial concentration of KY-109 was 100 μg/ml.

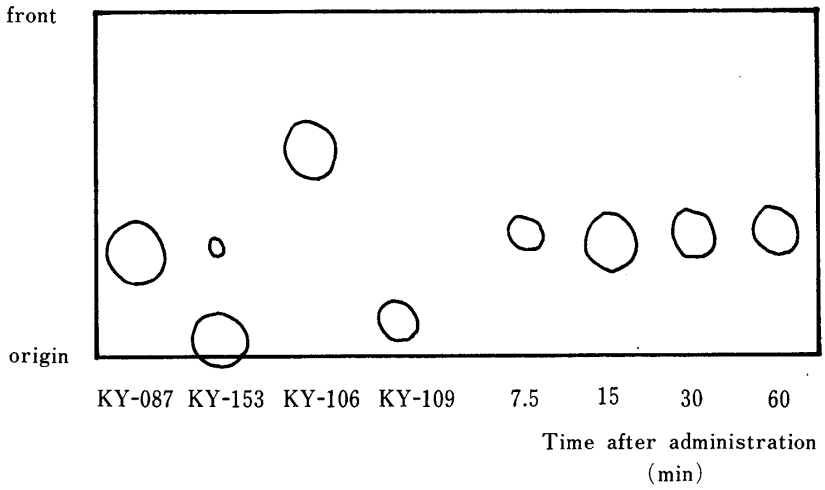


Fig. 5. Bioautograms of Portal Blood after Oral Administration of KY-109 in Rats at a Dose of 100 mg/kg

Adsorbent: Silica gel 60 (Merck), 2×20 cm. Solvent: chloroform–ethanol–formic acid (10:2:1). Test organism: *Bacillus subtilis* ATCC 6633.

77.7% in the homogenates of the stomach, small intestine, cecum and colon, respectively, after 30 min of incubation.

Active Metabolites in Portal Blood

A bioautogram of the portal blood after oral administration of KY-109 to rats is shown in Fig. 5. Only a single active spot could be detected in the portal blood at 7.5, 15, 30 and 60 min after administration. The spot coincided with that of authentic KY-087.

Discussion

It is well known that a prodrug to overcome the problem of poor oral absorption must

satisfy three important criteria.[3,4] First, it must be dissolved in the gastrointestinal fluids. Second, it must be transported across the gastrointestinal membrane. Third, it should revert to the parent drug at the right time and place.

In this study, KY-087 (the parent drug) was poorly absorbed orally in rats, probably because of its low lipophilicity; the values of lipophilicity (1-octanol-H_2O, pH 6.5) of KY-087, KY-106 and KY-109 were 0.0098, 39.0 and 7.8, respectively.[1] KY-153, which has only a hydrophilic promoiety, was also poorly absorbed. It is considered that this poor absorption may be attributable to low lipophilicity or rapid hydrolysis to KY-087 in the gastrointestinal tract.

When KY-106, which has only a lipophilic promoiety, was administered orally as a 0.5% MC suspension, it was hardly absorbed. However, when KY-106 was dissolved in 50% propylene glycol and administered orally, the absorption was remarkably improved. Thus, the incomplete absorption of KY-106 when administered orally as a 0.5% MC suspension is considered to be attributable to its poor solubility in the gastrointestinal fluid. From this result, it can be assumed that KY-106 itself is essentially quite readily absorbable. This result is in good agreement with the findings reported by Wright *et al.*[5] with regard to the acetoxymethyl esters of cefamandole, which is similar in structure to KY-106.

KY-109, in which, in addition to the 4-carboxyl ester, the side-chain α-hydroxy group has been esterified with L-alanine (containing a hydrophilic amino group), combines an increased degree of lipophilicity with good aqueous solubility. KY-109, when administered orally to rats, was well absorbed, and the serum level was nearly equal to that after administration of KY-106 in 50% propylene glycol. The absorption site of KY-109 was clarified to be the upper small intestine, since it was found that KY-109 was absorbed mainly from the upper small intestine in rats.

KY-109 was stable in the stomach contents, but was easily hydrolyzed in the contents of the small intestine, cecum and colon. The major hydrolytic product was KY-106. KY-109 was also found to be mainly hydrolyzed to KY-087 *via* KY-106 in the gastrointestinal homogenate.

When KY-109 was administered orally to rats, only the hydrolysis product KY-087 could be detected in the portal blood. This result indicates that KY-109 is completely hydrolyzed to KY-087 before passing into the portal blood.

Form the above observations, the absorption mechanism of KY-109 can be speculated to

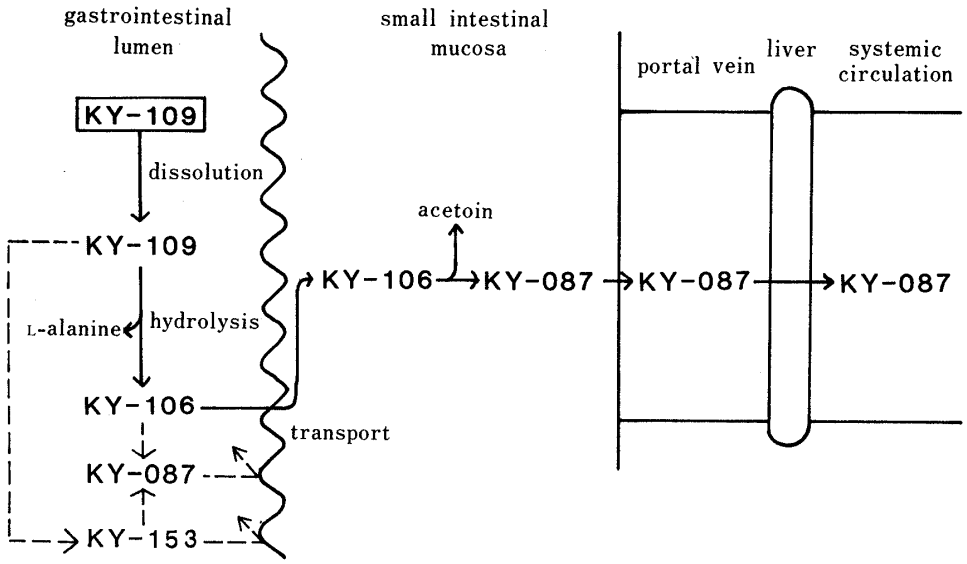


Fig. 6. Proposed Intestinal Absorption Mechanism of KY-109

be as illustrated in Fig. 6. KY-109 is transferred into the small intestinal lumen without hydrolysis in the stomach after oral administration, and is then hydrolyzed to KY-106 with the release of the L-alanyl residue. The resulting KY-106 is efficiently transferred into the mucosal membrane, and hydrolyzed enzymatically with the formation of both KY-087 and acetoin,[6] which is contained in natural dairy products, *etc.* The resulting KY-087 is then transferred into the systemic circulation through the portal vein and liver.

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