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In vitro Acetolactate Synthase Inhibition of LGC-40863 in Rice and Barnyardgrass

Bae, Y.T., J.H. Lee, and S.J. Koo*

신규제초제 LGC-40863의 벼와 피에 대한 Acetolactate synthase 저해 활성

배영태 · 이재환 · 구석진*

ABSTRACT

LGC-40863(proposed common name ; pyribenzoxim), (benzophenone *O*-[2,6-bis[(4,6-dimethoxy-2-pyrimidinyl)oxy]benzoyl]oxime) is a new rice herbicide being developed by LG Chemical Ltd. The herbicide is highly selective between rice(*Oryza sativa* L.) and weeds including barnyardgrass (*Echinochloa crus-galli*(L.) P. Beauv.), and assumed to inhibit acetolactate synthase(ALS ; EC 4.1.3.18) because other structurally related herbicides inhibit the enzyme. To know inhibitory activity and the mode of inhibition of LGC-40863, I_{50}(concentration inhibiting ALS activity by 50%) and inhibition kinetics were investigated using ALS extracted from rice and barnyardgrass. I_{50} values of LGC-40863 were 14 and 16mM in rice and barnyardgrass, respectively. In contrast to imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridine-carboxylic acid) which showed an uncompetitive inhibition pattern, LGC-40863 was a noncompetitive inhibitor to ALS with respect to pyruvate similar to chlorsulfuron(2-chloro-N-((4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl)benz-enesulfonamide) in both plants.

Key words ; LGC-40863, pyribenzoxim, acetolactate synthase, rice, barnyardgrass.

INTRODUCTION

LGC-40863 is a new rice herbicide being developed by LG Chemical Ltd. The herbicide controls various weeds including barnyardgrass and is highly safe to rice, wheat(*Triticum aestivum* L.), and turfgrass[8]. This chemical is categorized in pyrimidinyloxybenzoate herbicides, which include pyrithiobac[3,6,12], KIH-2023[14], and KIH-6127[5] developed for cotton or rice. Similar to sulfonylurea and imidazolinone herbicides, these chemicals were reported to inhibit ALS[5,12,14], the first common enzyme in the biosynthetic pathway of the branched-chain amino acids, valine, leucine, and isoleucine. Although the mode of action

* LG Chem Research Park, P.O. Box 61, YuSong, Science Town, Taejon, 305-380, KOREA

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of LGC-40863 has not been investigated, the symptom of the treated plants was similar to those of other ALS inhibitors. In this study, to understand whether LGC-40863 acts as an ALS inhibitor, *in vitro* inhibition activity(I_{50}) and the inhibition pattern were investigated with ALS extracted from rice and barnyardgrass. Chlorsulfuron and imazapyr, known ALS inhibiting herbicides[1,4,10], were also used for comparison.

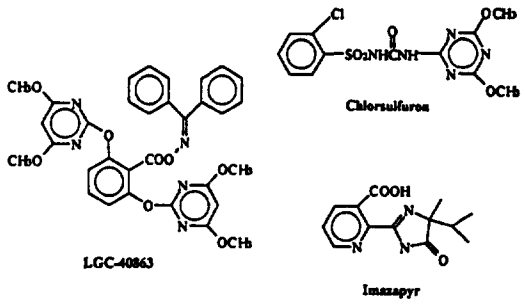

Fig. 1. Structure of ALS-inhibiting herbicides used for the present study.

MATERIALS AND METHODS

Plant material

Seeds of rice and barnyardgrass were planted in pots containing a sandy loam soil and grown for 2 weeks in a greenhouse kept at 35/25℃ (day/night). Shoots were harvested, rinsed in tap water and powdered in liquid nitrogen with a mechanical homogenizer.

ALS extraction

ALS of rice and barnyardgrass was extracted by the method of Ray[9] with slight modifications. The powdered shoot sample was mixed with PVPP(Polyvinylpolypyrrolidone), and homogenized in twice the volumes of extraction buffer(20mM K-phosphate at pH 7.5, 10% glycerol, 0.5mM $MgCl_2$, 0.5mM thiamine-pyrophosphate, 10mM FAD, 1mM DTT, 1mM pyruvate and 100mM ascorbic acid). The homogenate was filtered through 8 layers of cheesecloth, and the filtrate was centrifuged at 27000x*g* for 20min at 4℃. The supernatant was precipitated in 25 to 50% of ammonium sulfate, and centrifuged to harvest the pellet. The pellet was re-dissolved in minimum volume of desalting buffer(20mM K-phosphate at pH 7.5, 10% glycerol, 0.5mM $MgCl_2$, 0.5mM thiamine-pyrophosphate, 10mM FAD, 1mM DTT). This solution was passed through a Sephadex G-25 column(Pharmacia PD-10) equilibrated with the same buffer. The fraction containing the desalted protein was collected and used for further studies. All procedures were carried out at 4℃.

ALS Assay

The final volume of the reaction solution was 0.5ml, and the reaction solution contained 20mM K-phosphate at pH 7.0, 0.5mM $MgCl_2$, 0.5mM thiamine-pyrophosphate, 10mM FAD, 20mM pyruvate, an inhibitor at 0, 3.9, 7.8, 15.6, or 31.3mM(LGC-40863 and imazapyr), or at 0, 7.8, 15.6, 31.3, or 62.5nM(chlorsulfuron). The reaction was initiated by adding 50ml of the enzyme solution, and terminated by adding 50ml of 6N H_2SO_4 after incubation at 30℃ for 30min. Acetolactate was determined by the method of Westerfeld[13] with slight modifications. The reaction solutions were heated at 60℃ for 15min, and 0.5ml of 0.5%(w/v) creatine was added. Then, 0.5ml of 5%(w/v) -naphthol, freshly prepared in 2.5N NaOH, was added, and the solutions were heated for further 15min at 60℃. The colorated solutions were centrifuged at 16000x*g* for 5min, and absorbance of the supernatants was determined at 530nm. I_{50} values were calculated by the equation of Ray[9] ; %Activity = 100/(1 + Inh/I_{50}), where Inh is the inhibitor concentrations.

RESULTS AND DISCUSSION

The I_{50} values of LGC-40863 were 14 and

Table 1. I_{50} values of LGC-40863, chlorsulfuron, and imazapyr to acetolactate synthase each extracted from rice and barnyardgrass.

ALS from	LGC-40863	Chlorsulfuron	Imazapyr
	mM		
Rice	14	0.033	12
Barnyardgrass	16	0.043	15

* I_{50} : Concentration inhibiting ALS activity by 50%.

16mM for rice and barnyardgrass, respectively (Table 1). These values were similar to those of imazapyr, but about 400 times higher than those of chlorsulfuron. Sensitivity of ALS did not differ greatly between rice and barnyardgrass to LGC-40863 as well as to chlorsulfuron and imazapyr. However, in whole plant responses, LGC-40863 was highly selective between two species[8]. The I_{50} data obtained from the present study suggest that selectivity of LGC-40863 is not based on differential selectivity at ALS enzyme. Therefore, selectivity of LGC-40863 may be based on differences of uptake, translocation, or metabolism between crops and weeds. Previous studies with sulfonylurea and imidazolinone herbicides have shown that metabolic differences were important in selectivity[7].

Lineweaver-Burk plots show that LGC-40863 is a noncompetitive type inhibitor for ALS with respect to pyruvate in both rice and barnyardgrass (Fig. 2, Fig. 3). Similar to the previous studies with other plants[1,4,10], chlorsulfuron and imazapyr showed a noncompetitive and uncompetitive inhibiton pattern, respectively, in rice(Fig. 4, Fig. 5). Therefore, the inhibition pattern of LGC-40863 was similar to that of chlorsulfuron. Since Kis is not equal to Kii, both LGC-40863 and chlorsulfuron seem to be mixed-type noncompetitive inhibitors. In mixed-type noncompetitive inhibition, although the inhibitor does not compete with pyruvate for the binding site, the binding affinity of substrate to the enzyme may be influenced by

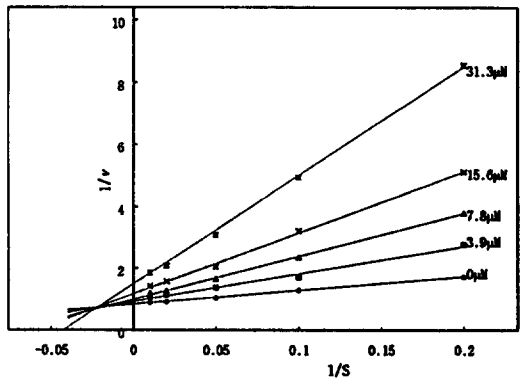

Fig. 2. Lineweaver-Burk plot of LGC-40863 for barnyardgrass acetolactate synthase ; Kis=4.5μM, Kii=5.01μM.

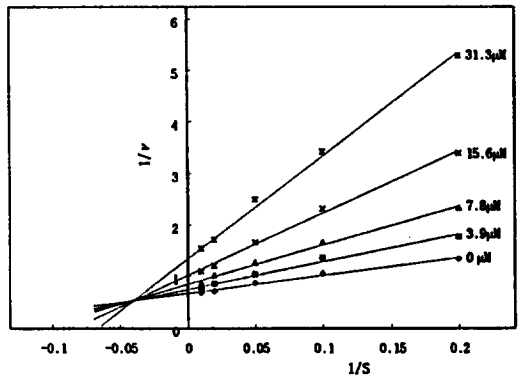

Fig. 3. Lineweaver-Burk plot of LGC-40863 for rice acetolactate synthase ; Kis=6.5μM, Kii=29μM.

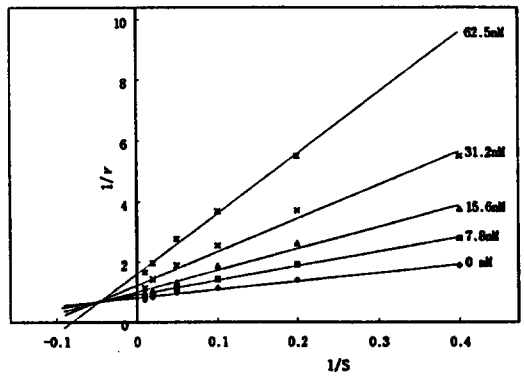

Fig. 4. Lineweaver-Burk plot of chlorsulfuron for rice acetolactate synthase ; Kis=10.2nM, Kii =58.9nM.

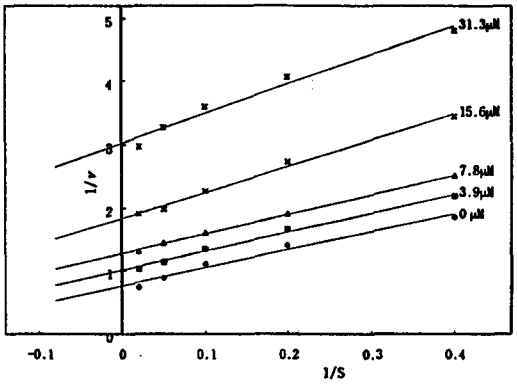

Fig. 5. Lineweaver-Burk plot of imazapyr for rice acetolactate synthase ; Ki=9.8μM.

the inhibitor. Durner *et al.*[4] suggested that the binding site of chlorsulfuron seemed to overlap with the domain of the second pyruvate. Since LGC-40863 showed similar mixed-type noncompetitive inhibition, the mode of binding of the herbicide to ALS may be similar to that of chlorsulfuron. By comparison, imazapyr showed uncompetitive inhibition for rice ALS as reported by Shaner *et al.*[10] in other plants. They suggested that imazapyr might bind to the ALS-pyruvate complex[11].

The I_{50} values(14 to 16 mM) of LGC-40863 (Table 1) are about 1,000 times higher than that of pyrithiobac(15nM)[12]. However, the use rates of the two structurally-related herbicides seem to be similar[8,12]. It was shown from the structure-activity studies that the methyl ester of pyrimidinyloxybenzoate was inactive even at mM concentrations *in vitro*, suggesting the free carboxyl moiety is essential for ALS inhibition of pyrimidinyloxybenzoate herbicides. Since LGC-40863 is an oxime-substituted compound on the carboxyl group, and its I_{50} values are considerably higher than the unsubstituted molecules, it is possible to speculate that LCG-40863 may be a pro-herbicide, and activated by hydrolysis at the oxime bond in the plants. Nevertheless, because LGC-40863 showed the I_{50} values similar to imidazolinone herbicides, and the distinct kinetics patterns, the possibility that LGC-40863 itself is the active herbicidal compound is not excluded. Plant metabolism of LGC-40863 needs to be investigated to understand whether an active form is involved in herbicidal action.

적 요

(주)LG화학에서 개발중인 LGC-40863은 벼에 대해 높은 안전성을 보이며 피 등 여러 잡초를 방제하는 신규제초제이다. LGC-40863은 관련 제초제나 식물에서의 효과 발현 증상으로부터 acetolactate synthase(ALS)를 저해하는 것으로 추정된다. 본 연구에서는 LGC-40863의 ALS에 대한 영향을 알아보기 위하여 벼와 피로부터 추출한 ALS를 사용하여 I_{50}값과 효소반응속도를 조사하였다. LGC-40863의 I_{50}값은 14 (벼), 16mM(피)였고, 대조약제로 사용한 imazapyr와 chlorsulfuron의 I_{50}값은 각각 12-15mM, 0.03-0.04mM였으므로 LGC-40863의 I_{50}값은 imazapyr와 유사하고 chlorsulfuron에 비해서는 약 400배 정도 높았다. 그리고 세 가지 약제 모두 벼와 피 간의 감수성 차이는 크지 않았다. 효소반응속도 면에서 살펴보면, LGC-40863은 벼와 피 모두 pyruvate에 대해 noncompetitive inhibition을 나타내었으며, chlorsulfuron과 imazapyr는 벼에서 각각 noncompetitive와 uncompetitive inhibition을 하는 것으로 나타났으므로 LGC-40863은 chlorsulfuron과 저해유형이 비슷하였다.

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