Chapter III

Results

1.Anticonvulsant activity

Anticonvulsant activities of the tested compounds were evaluated in mice using two primary standard screening models, MES and PTZ tests. While NSS and PEG 400 which were given to the control groups of mice, and exhibited no protection in both MES and PTZ models. Anticonvulsant activity of the tested compounds, ameltolide and CU-17-06 were demonstrated in MES but not in PTZ model.

1.1 Anticonvulsant activity against MES

As shown in figure 5 and 6 intraperitoneally given CU-17-06 and ameltolide demonstrated a protection against MES in mice in a dose dependent. The ED_{50} of CU-17-06 were 92.04, 77.62 and 118.85 mg/kg B.W. at pretreated times of 15, 30 and 60 min, respectively while corresponding values ameltolide were 1.11, 1.08 and 1.15 mg/kg B.W.

The optimal pretreated time defined as the minimal time for the test substance to exert its highest anticonvulsant activity was found to be 30 min in both CU-17-06 and ameltolide. As shown in Figure 8 the ED_{50} of CU-17-06 and ameltolide at optimal pretreated time were 77.62 and 1.08 mg/kg B.W. respectively.

1.1.1 Duration of protection against MES

The ED_{50} of intraperitoneally given CU-17-06 and ameltolide were demonstrated at 3 and 6 hours after dosing. The ED_{50} of both CU-17-06 and ameltolide increased as a function of time. At pretreated time of 1, 3 and 6 hours, the ED_{50} of CU-17-06 was 118.85, 257.03 and 467.73 mg/kg B.W. respectively. While corresponding values for ameltolide were 1.15, 3.54 and 10.0 mg/kg B.W. (Fig.7)

1.2 Anticonvulsant activity against PTZ

Like ameltolide, CU-17-06 does not demonstrated anticonvulsant activity against convulsion induced by PTZ, ameltolide in the dose up to 10 mg/kg B.W. does not protect any experimental animals in this model. CU-17-06 in the dose up to 600 mg/kg B.W. does not protect any experimental animals in this model either.

2. Toxicity

2.1 Lethality

The most frequent clinical signs observed in mice receiving high dose of ameltolide were ataxia, sedation, hypnosis and dyspnea. Lethality was observed within the period of 72 hours, however, death occurred mostly within 24 hours. The median lethal dose (LD_{50}) of ameltolide was 62.80 mg/kg B.W., respectively (Fig. 9). In case of CU-17-06, giving intraperitoneally up to 1,000 mg/kg B.W. does not show clinical signs of death in any experimental animals except sedation and hypnosis.

2.2 Rotarod test

In rotarod test, control mice, receiving NSS and PEG 400, were able to maintain their equilibrium for at least 1 min on the rotating rod in 3 successive trials. The neurological impairment as indicated by an ability of the animal to maintain their equilibrium was exhibited by an intraperitoneal administration of various doses ofCU-17-06 and ameltolide. As illustrated in Figure 10and 11 both CU-17-06 and ameltolide inhibited the rotarod performance in a dose- dependent manner. The TD_{50} of CU-17-06 and ameltolide at optimal pretreated time, 30 min, were 323.59 and 9.09 mg/kg B.W., respectively.

The protective index (PI= TD_{50}/ ED_{50}), which were 4.16 and 8.41 for CU-17-06 and ameltolide, respectively. (Table 7)

3. Effect on some cortical amino acid neurotransmitter levels in freely moving rats.

The excitatory neurotransmitters in question are glutamate and aspartate whereas GABA and glycine are the ones with inhibitory effect. Alteration of amino acid neurotransmitter' s levels was expressed as percentage of change from basal value, which was determined from three consecutive samples before the administration of the test substances. Qualitative and quantitative determination of the amount of the amino acids was accomplished by HPLC as exemplified by HPLC chromatogram in Figure 13 and 14.

In control groups, the effect of PEG 400 on spontaneous release of cortical aspartate, glutamate, glycine and GABA was not statistically from those of NSS (Fig. 19-22). Ameltolide in the dose of 1.1 and 2.2 mg/kg B.W. did not exert any significant effect on the levels of aspartate, glutamate, glycine and GABA. Similarity to ameltolide, CU-17-06 did not show any significant changing in both of excitatory amino acid neurotransmitter (aspartate and glutamate) and inhibitory amino acid neurotransmitter (glycine and GABA) in dose of 77.62 and 155.2 mg/kg B.W. (Fig. 15-18).

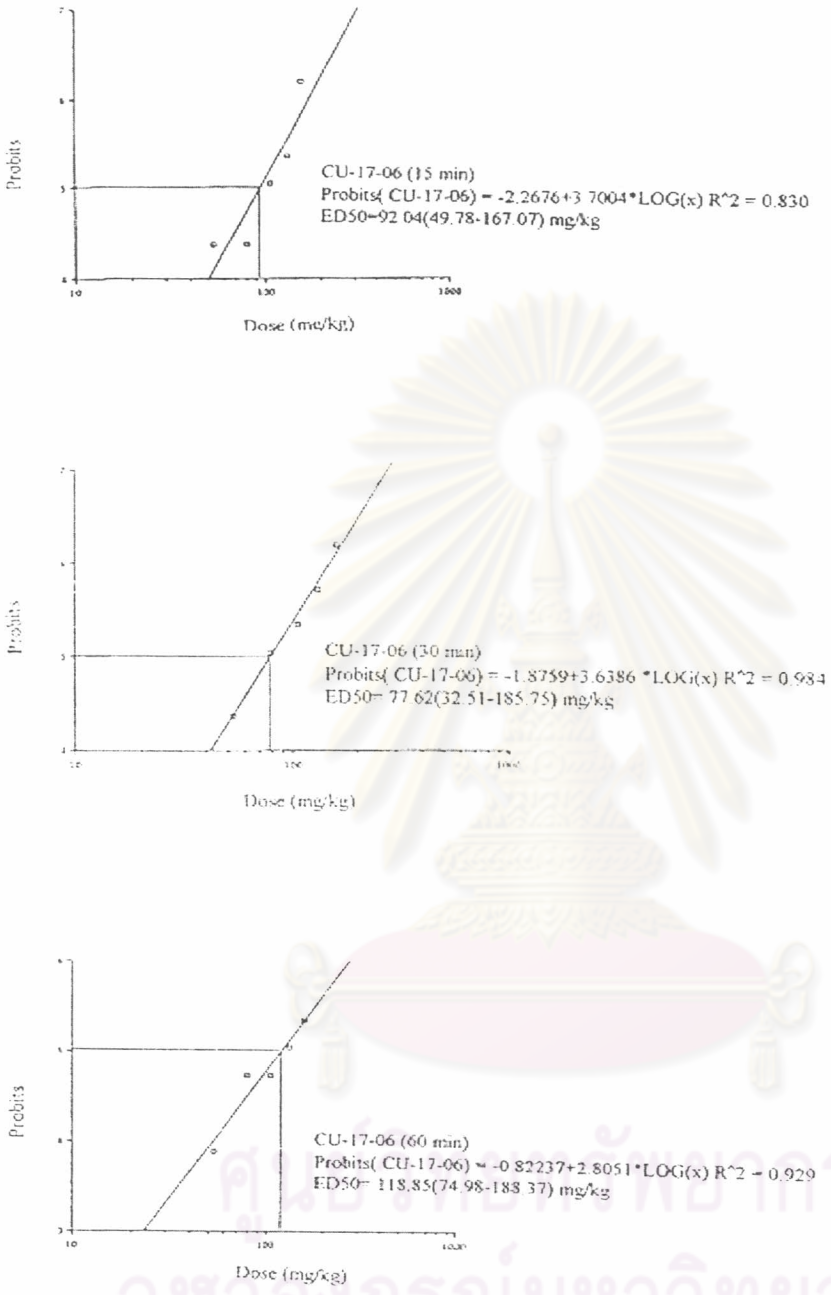


Figure 5 Log dose response curves of CU-17-06 (i.p.) against MES

in mice at 15, 30, and 60 min-pretreated times

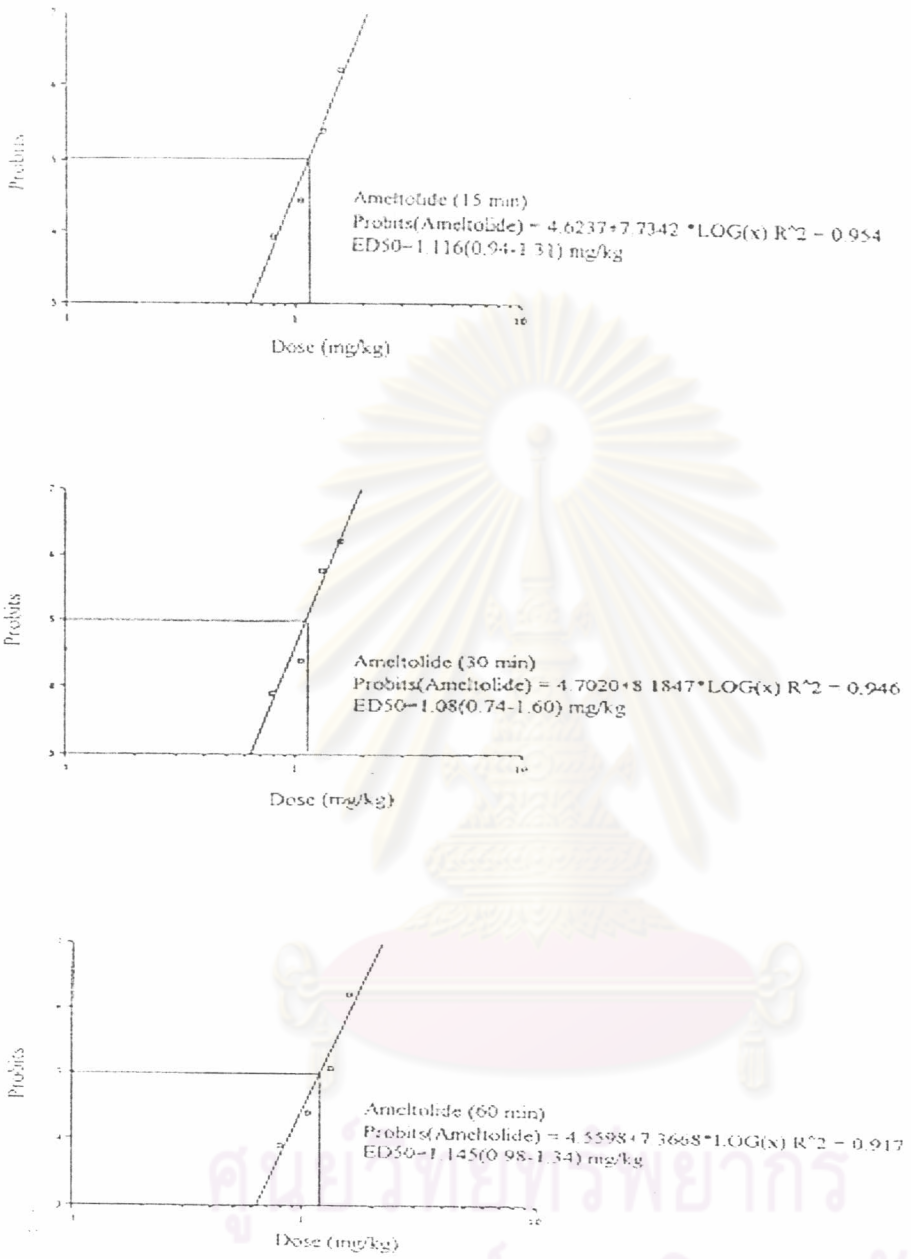


Figure 6 Log dose response curves of ameltolide (i.p.) against MES

in mice at 15, 30, and 60 min pretreated times

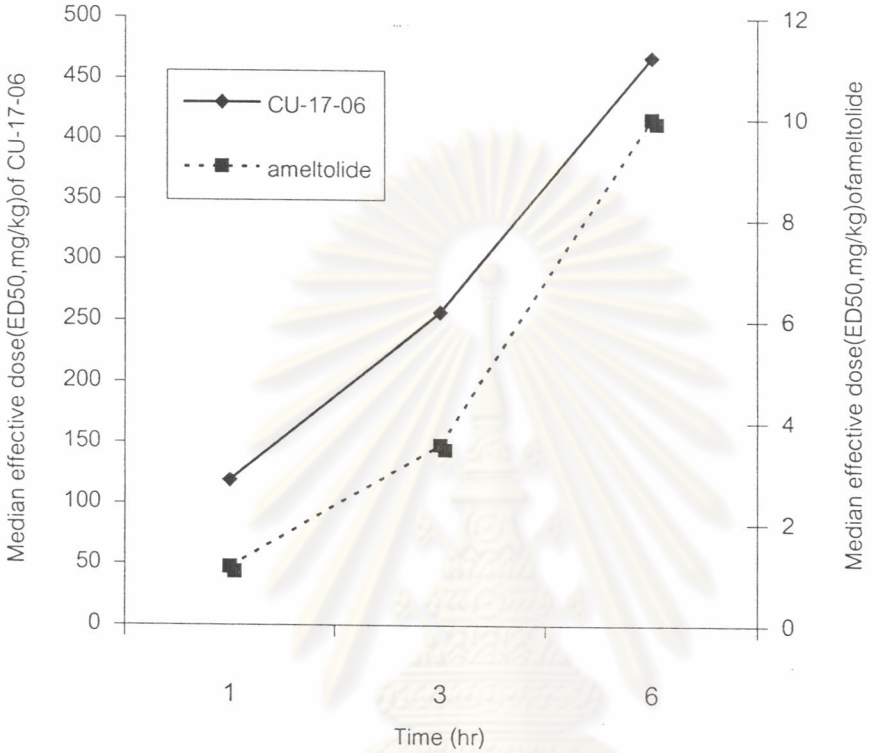


Figure 7 Protection against MES exhibited by CU-17-06 and ameltolide at various pretreated times in mice

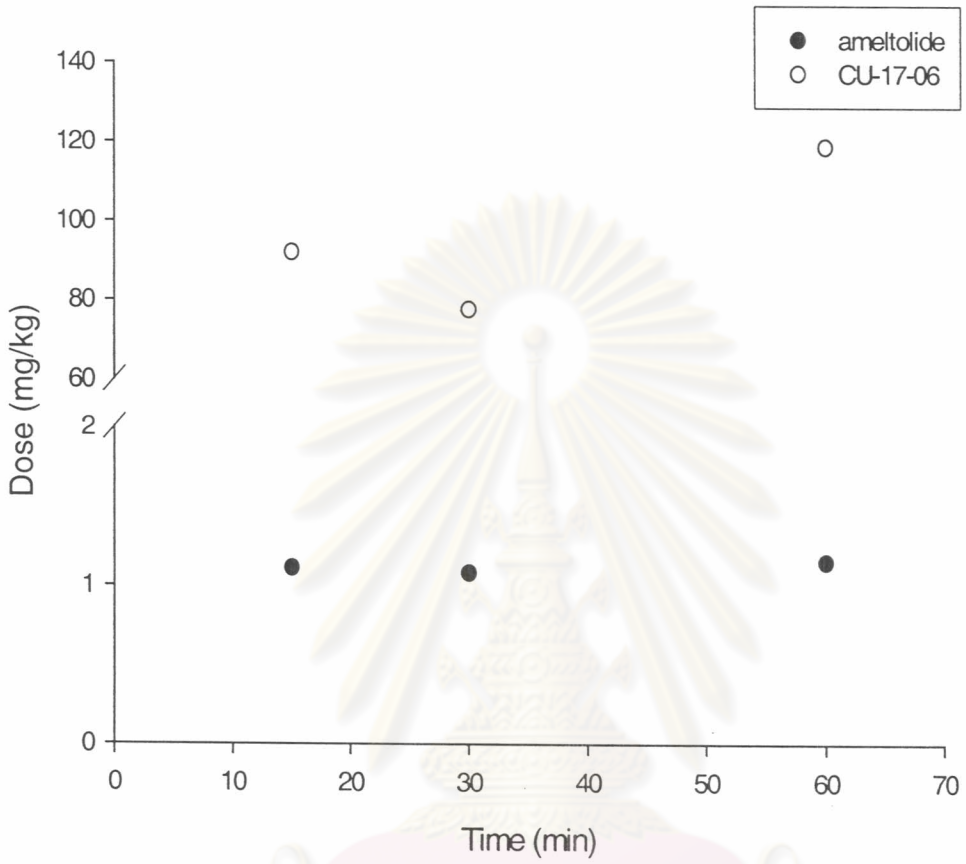
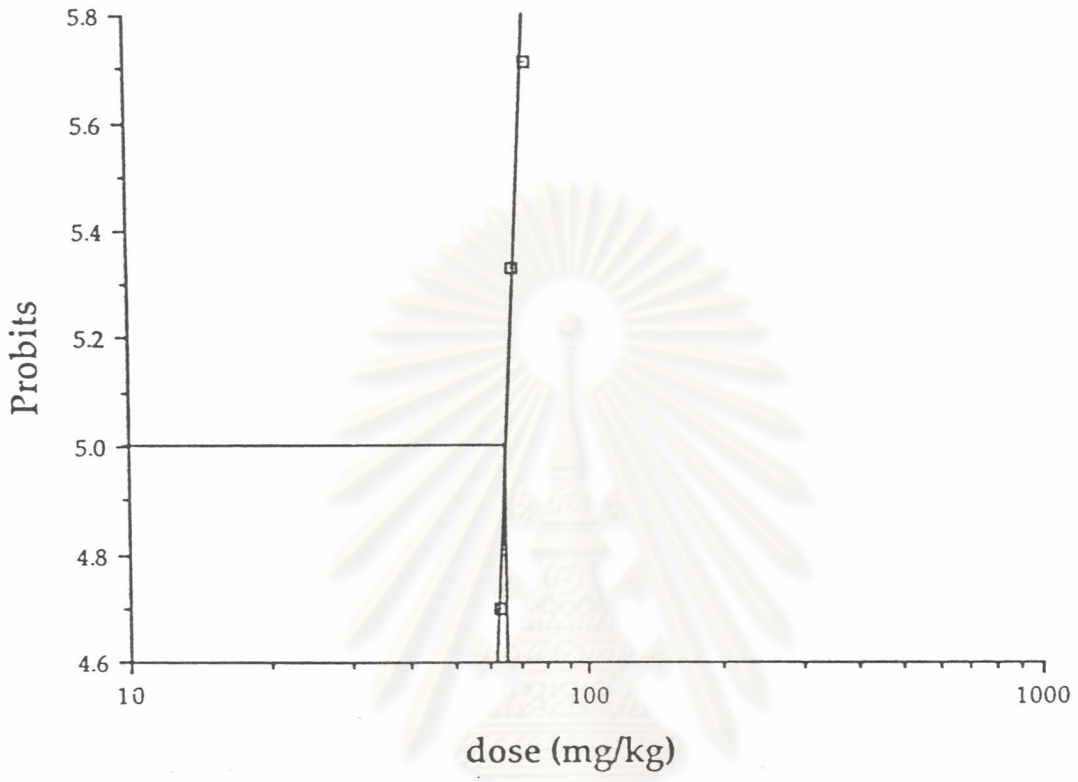


Figure 8 Comparison of ED_{50} at various pretreated times of CU-17-06 and ameltolide (i.p.) in mice

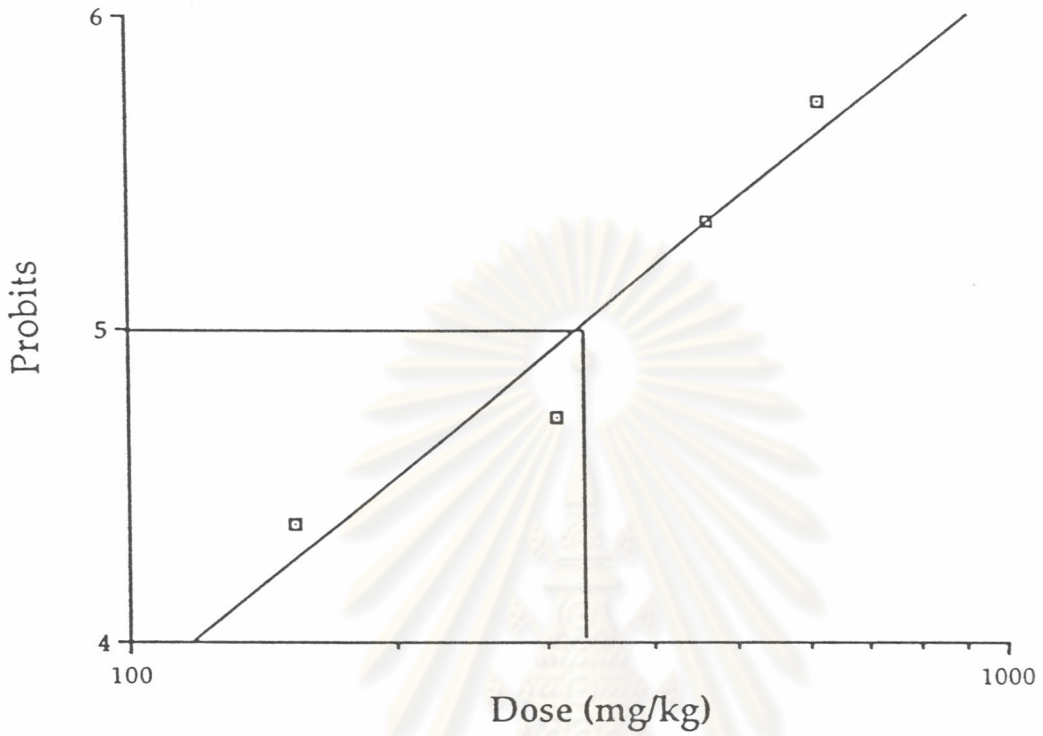


Probits (Ameltolide) = -22.184+15.127* LOG (x) R ^ 2=0.986

LD50=63.09 mg/kg

Figure 9 Log dose response curves of acute toxicity (lethality)

of ameltolide (i.p.) in mice

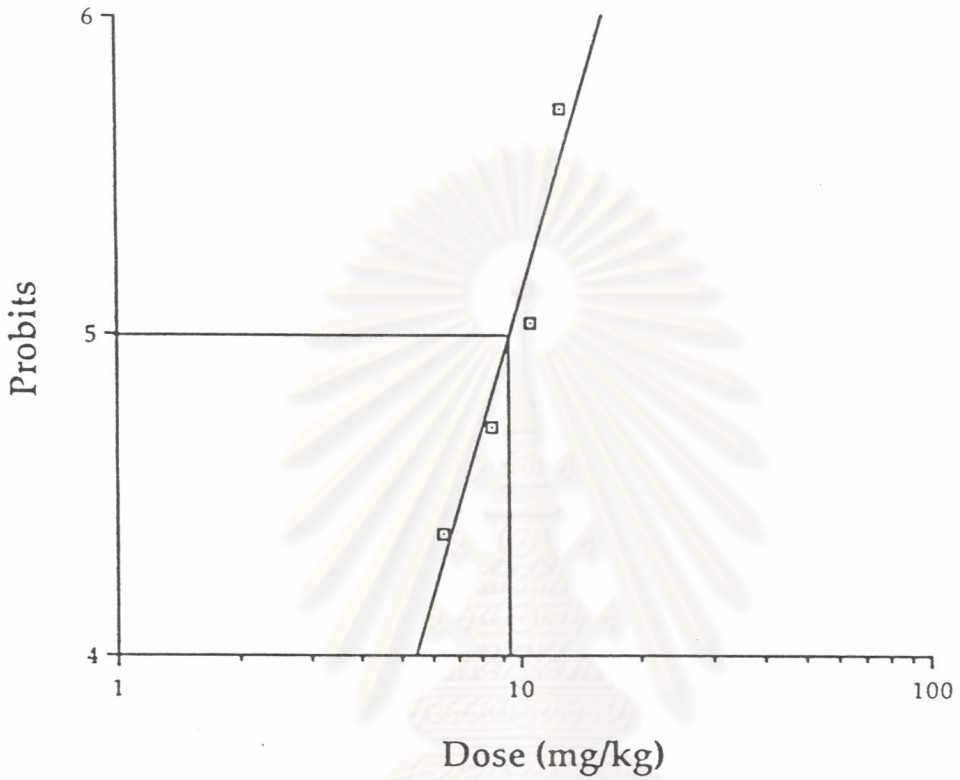


Probits (CU-17-06) = -0.66741+2.2499* LOG (x) R ^ 2=0.933

TD50=323.59 mg/kg

Figure 10 Log dose response curve of neurotoxicity (Rotarod test)

exhibited by CU-17-06(i.p.) in mice



Probits (Ameltolide) = 0.97973+4.1940* LOG (x) R ^ 2=0.923

TD50=9.099mg/kg

Figure 11 Log dose response curve of neurotoxicity (Rotarod test) exhibited by ameltolide (i.p.) in mice

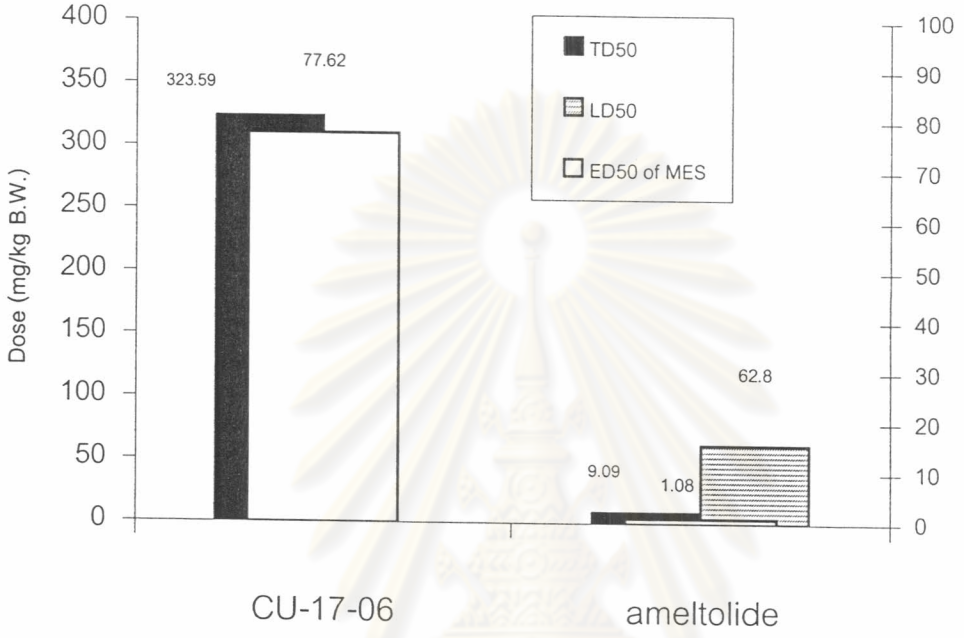
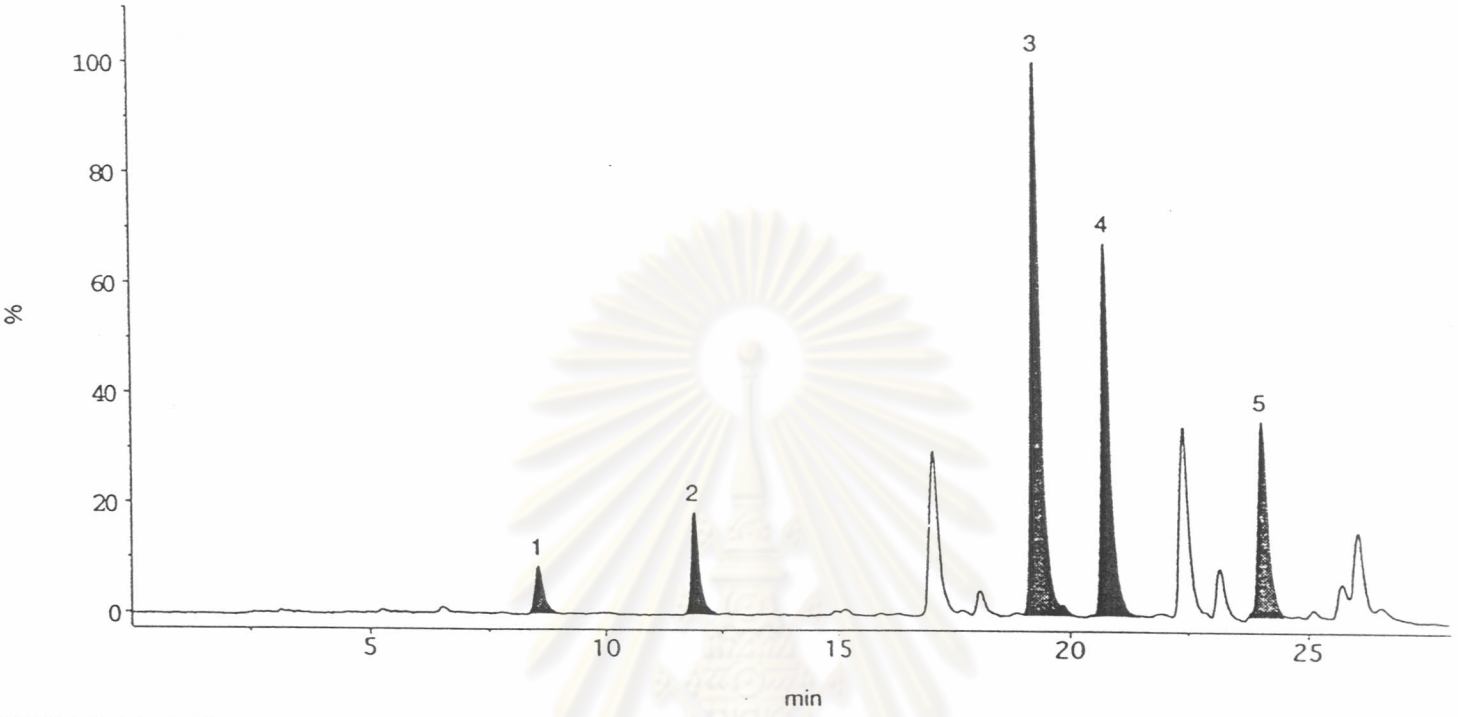


Figure 12 Illustration of the LD_{50}, $TD_{50,}$ and ED_{50} elicited by an intraperitoneal administration of CU-17-06 and ameltolide in mice in MES model

* LD_{50} of CU-17-06 could not be determined (no lethality in the dose of 1,000 mg/kg)

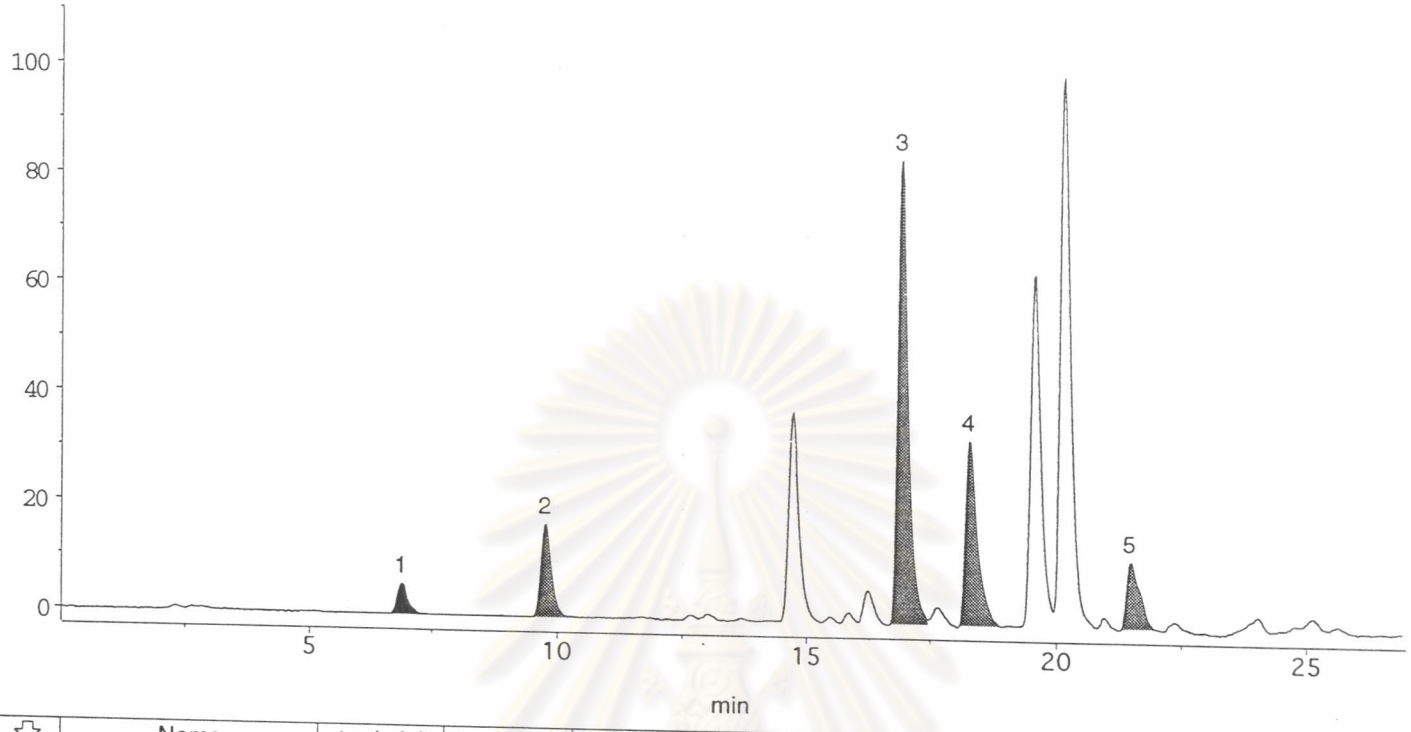
Table 7 ED_{50}, TD_{50}, LD_{50}, PI (TD_{50}/ ED_{50}) and relative safety margin (LD_{50}/ ED_{50}) of an intraperitoneal administration of CU-17-06 and ameltolide in MES and PTZ models

Parameter	Animal model	CU-17-06	Ameltolide
ED_{50} (mg/kg)	MES	77.62 (32.51-185.75)	1.08 (0.74-1.60)
	PTZ	No protection	No protection
TD_{50}(mg/kg B.W.)	Rotarod	320	9
PI	MES	4	9
	PTZ	–	–
LD_{50}(mg/kg B.W.)	-	>1,000	63
Relative safety margin	MES	> 13	63
	PTZ	–	–



Peak		Name	t_R (min)	Start (min)	End (min)	Area	Height (%)	Norm (%)	Peak Type	Amount
1		ASP	8.525	8.350	9.025	1.990	5.40	3.67	BB	
2		GLU	11.900	11.675	12.425	4.189	11.51	7.72	BB	
3		HOMO	19.275	19.000	20.125	23.811	62.20	43.87	BB	
4		GLY	20.750	20.375	21.475	15.961	42.00	29.41	BB	
5		GABA	24.050	23.750	24.500	8.323	35.51	15.33	BB	
						54.27	156.61	100.00		

Figure 13 HPLC chromatogram of (ameltolide)OPA-derivatized amino acids from the rat cerebral cortex



Peak		Name	t_R (min)	Start (min)	End (min)	Area	Height (%)	Norm (%)	Peak Type	Amount	F
1		ASP	6.875	6.225	9.525	1.31	2.114	3.56	BD		
2		GLU	9.775	9.525	10.350	3.876	6.597	10.52	DB		
3		HOMO	16.925	16.725	17.450	19.559	32.986	53.10	BB		
4		GLY	18.275	18.100	18.875	8.760	13.095	23.78	BB		
5		GABA	21.475	21.325	21.950	3.328	4.630	9.03	BB		
						36.83	59.422	100.00			

Figure 14 HPLC chromatogram of (CU-17-06)OPA-derivatized amino acids

from the rat cerebral corte

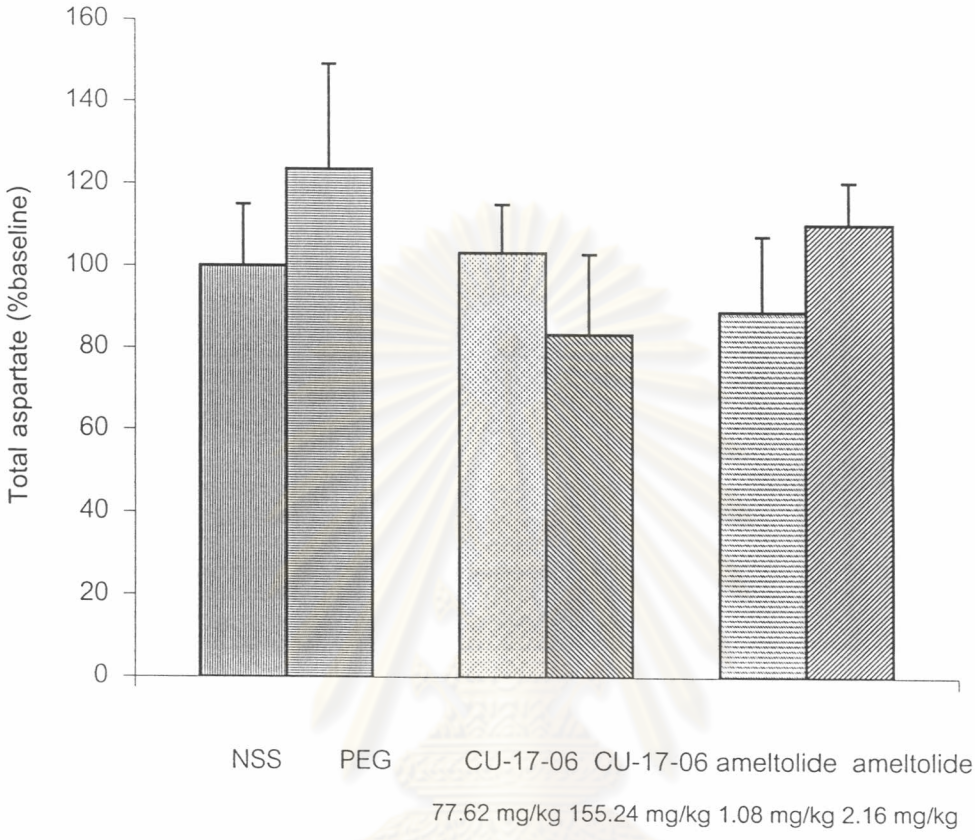


Figure 15 Effect of an intraperitoneal administration of CU-17-06 and ameltolide on the total amount of the rat cortical aspartate levels in the dialysate collected for 3 hours

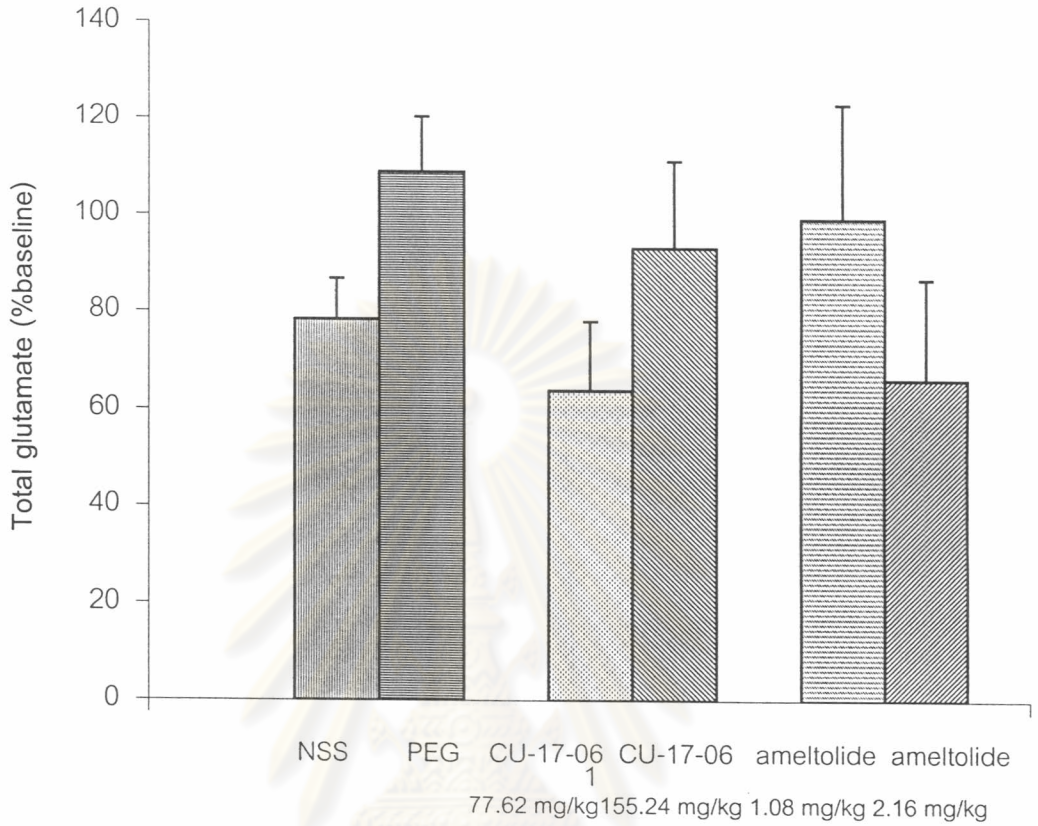


Figure 16 Effect of an intraperitoneal administration of CU-17-06 and ameltolide on the total amount of the rat cortical glutamate levels in the dialysate collected for 3 hours

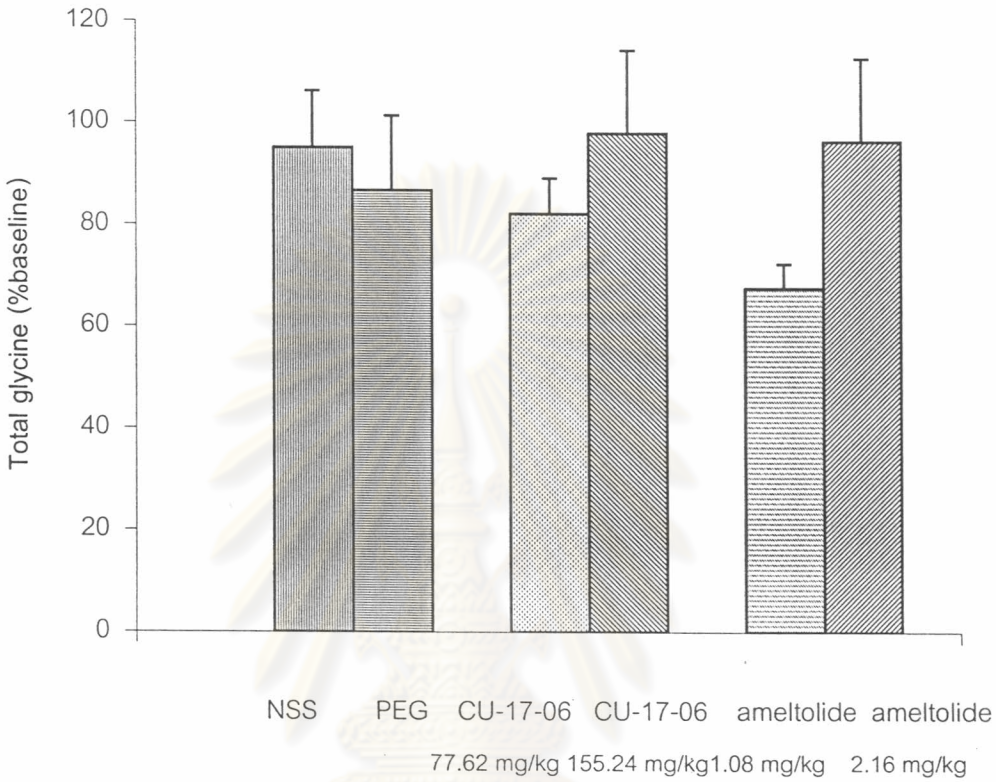


Figure 17 Effect of an intraperitoneal administration of CU-17-06 and ameltolide on the total amount of the rat cortical glycine levels in the dialysate collected for 3 hours

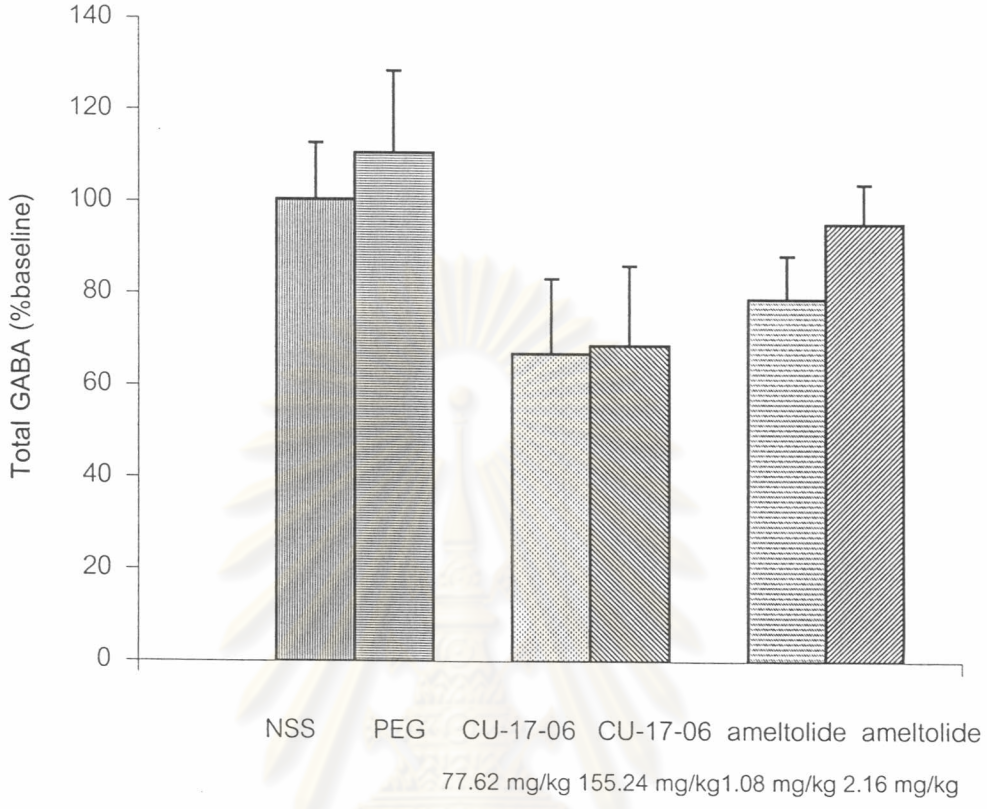


Figure 18 Effect of an intraperitoneal administration of CU-17-06 and ameltolide on the total amount of the rat cortical GABA levels in the dialysate collected for 3 hours

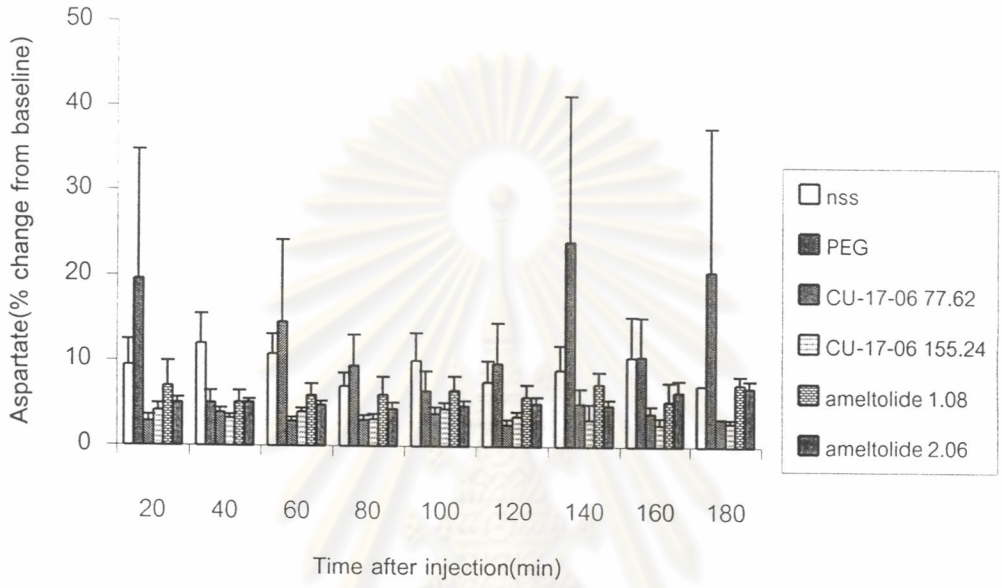


Figure 19 Effects of an intraperitoneal administration of CU-17-06 and ameltolide on the rat cortical aspartate levels at various times.

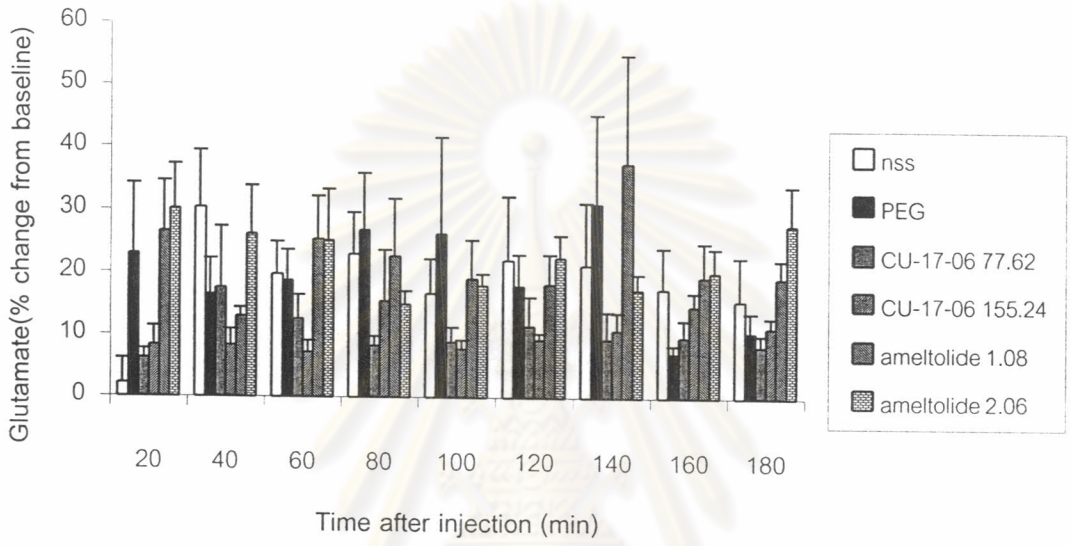


Figure 20 Effects of an intraperitoneal administration of CU-17-06 and ameltolide on the rat cortical glutamate levels at various times.

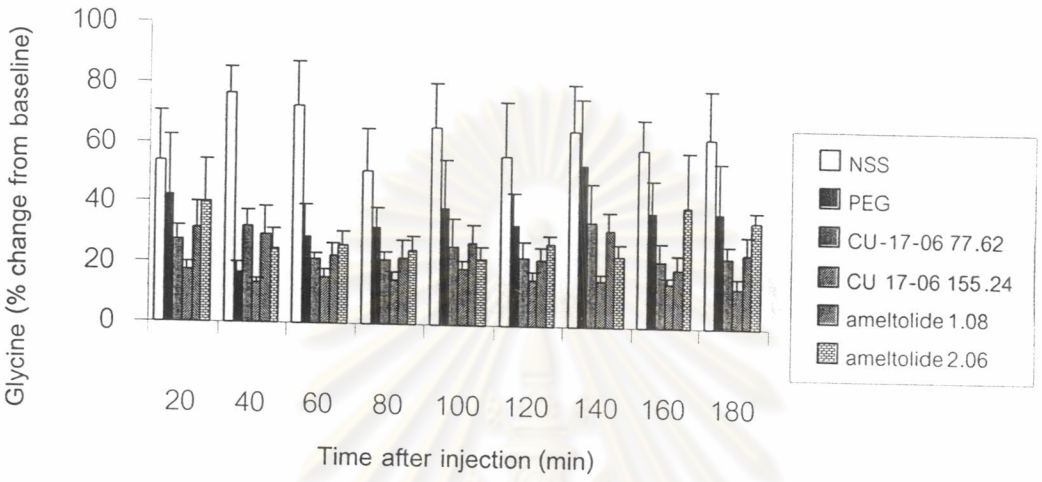


Figure 21 Effects of an intraperitoneal administration of CU-17-06 and ameltolide on the rat cortical glycine levels at various times.

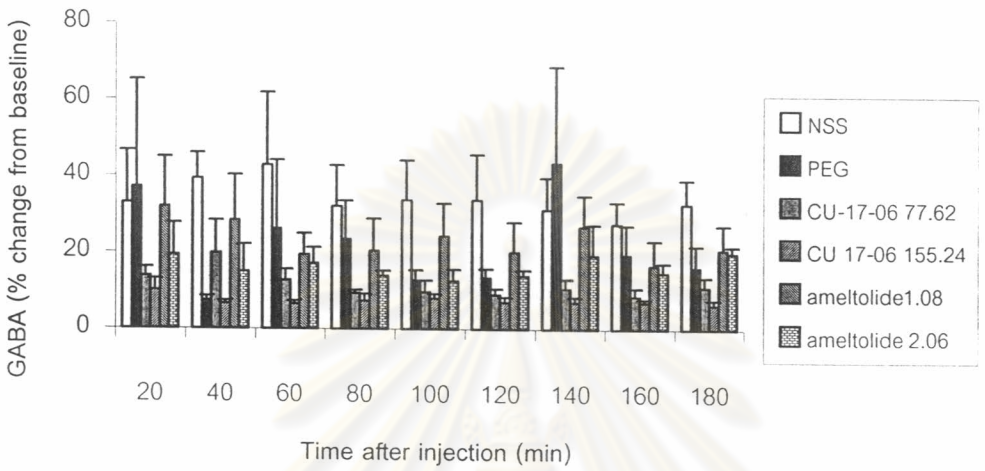


Figure 22 Effects of an intraperitoneal administration of CU-17-06 and ameltolide on the rat cortical GABA levels at various times.