constant or increasing dose of morphine. For example, the administration of phenobarbital increased aminopyrine N-demethylation by 199%, 257% and 285%, in controls, morphine (constant dose) treated rats and morphine (increasing dose) treated rats, respectively.

Moreover, the activity of N-methylbarbital N-demethylation increased by 435%, 393% and 533% and activity of hexobarbital hydroxylation increased by 160%, 202% and 225% in controls, morphine (constant dose) treated rats and morphine (increasing dose) treated rats, respectively. These results are not in accord with the observation made on male rats that the chronic administration of morphine abolished the stimulatory effect of phenobarbital on the microsomal drug-metabolizing enzymes.

Kato and Gillette have shown that the drug-metabolizing enzymes of liver microsomes of male rats which are stimulated by the male sex hormone are likely unstable and they easily lose their activity under abnormal physiological conditions.[5,6,13]

Moreover, Kato and Takayanagi recently reported that the administration of morphine depressed the activities of drug-metabolizing enzymes of liver microsomes only in male rats and it did not depress the activities in female rats and female and male mice, guinea-pigs and rabbits.

From these results it may be assumed that the abolishment of the stimulatory effect of phenobarbital in morphinized male rats is likely due to an intereferance of the stimulatory action of male sex hormone and it is not likely due to direct interferance on the stimulatory action of phenobarbital.

13) R. Kato, J.R. Gillette: J. Pharmacol., **150**, 279 (1965).

[Chem. Pharm. Bull. 15(9)1422~1426(1967)]

UDC 615.721-03

Mitsuo Mizutani and Shun-ichi Naito*1: Studies on Absorption and Excretion of Drugs. XXIX.*2 Biopharmaceutical Studies on Guaiacol Glycerol Ether and Related Compounds. I. Blood Level of Guaiacol Glycerol Ether in Rabbit and Its Binding with Serum Proteins.

(*Kyoto College of Pharmacy*[*1])

(Received August 6, 1966)

Recently, the pharmacological studies of Guaiacol Glycerol Ether (GGE) and its related compounds has been studied by Yamada,[1] Fujimura,[2] *et al.*

Little work has been done on biopharmacy of GGE, while there exist considerable literatures. Morgan[3] had reported plasma level of GGE after oral administration in the dog.

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*2 Part XXVIII: Yakuzaigaku, **26**, 145 (1966).

1) H. Yamada, S. Shibata, E. Narusawa: Nippon Yakurigaku Zasshi, **53**, 165 (1957).

2) H. Fujimura: Presented at 17th Nippon Yakugakkai Kinki-shibu Assembly (1961).

3) A.M. Morgan, E.B. Trruitt, J.M. Little: J. Am. Pharm. Assoc., **46**, 374 (1957).

Naito, one of the authors, reported some interactions of GGE with several additives, such as analgesics[4,5] and sulfonamides,[6] and also effects of GGE on serum cholesterol,[7,8] deposition of cholesterol in organs,[9] excretion of cholesterol[10] and blood sugar,[11] while those mechanisms has not yet known.

The following experiments were carried out to determine blood levels of GGE and its binding with serum proteins, as the first step of the biopharmaceutical studies on GGE and related compounds.

Experimental

Procedure——Suspensions were prepared by shaking finely powdered materials (screened through 100 mesh) with water without any suspending agent.

The resulting suspensions were administered to healthy male rabbits by intubation, before feeding in the morning for each sample at doses of 250 mg./kg., 375 mg./kg., and 500 mg./kg. of GGE. Blood samples were then taken at half an hour, 1, 2, 4, and 7 hr. None of food except water was given until the end of the experiment.

Determination of GGE——Four milliliters of 10% trichloroacetic acid solution was added to 1.0 ml. of plasma in 10 ml. glass-stoppered test tube and centrifuged for 5 minutes. The solution was filtered off and the filtrate was assayed spectrophotometrically in ultraviolet regin.

UV λ_{max} (log ε)=272 mμ (Fig. 1).

Blank plasma samples were assayed for appropriate corrections.

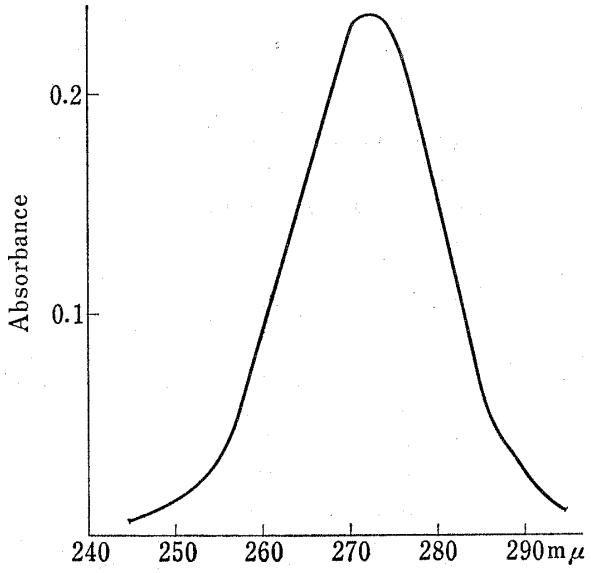

Fig. 1. Ultraviolet Absorption Spectrum of GGE in 10% Trichloroacetic acid (20 μg./ml.)

Binding of GGE with Rabbit Serum Proteins *in vivo*——Blood was taken at 1 and 2 hr., after the suspensions (500 mg./kg. of GGE) were administered to rabbits by intubation. Serum concentration of GGE (Ca), protein free, was determined by spectrophotometry.

Equilibrium Dialysis of GGE——The experimental design was followed by the method of Nakagaki, *et al.*[12] Eight milliliters of phosphate buffer solution (pH 7.4) was put into the outside of Visking Cellulose Tube in the standard tapered test tube and the mixture of 1.0 ml. of centrifuged rabbit serum (with citric acid) and 0.5 ml., 1.0 ml. and 2.0 ml. of phosphate buffer solution (pH 7.4), or without any buffer solution were used in the inside of bag, and then dialyzed at 4° for 72 hr. (see also Table I). It was verified by spectrophotometric determination that an aqueous solution of GGE is stable at least 72 hr. Guaiacol Glycerol Ether (Cb) in the outside of bag solution was determined spectrophotometrically.

The rate of binding of GGE with serum proteins (*in vivo*) was calculated from, $(Ca-Cb/Ca)\times 100\%$.

Binding of GGE with Rabbits and Human Serum Proteins *in vitro*——The experimental procedure was the same as in the case of *in vivo* principally. Aliquot of 8.0 ml. of phosphate buffer solution (pH 7.4) containing 1×10^{-3}, 5×10^{-4}, 1×10^{-4} M/L. of GGE, respectively, was put into the outside of bag. Aliquot of 3.0 ml. of phosphate buffer solution (pH 7.4) containing 1.48, 2.96% human serum proteins or 1.44, 2.88% of rabbit serum proteins was put into the inside of Visking Cellulose Tube (as shown in Table II). Solution of the mixture was dialyzed at 4° for 72 hr. Guaiacol Glycerol Ether was determined in the outside of bag solution. Blank serum were assayed for appropriate corrections.

4) S. Naito, A. Maeda: Kyoto Yakka Daigaku Gakuho, **12**, 17 (1964).
5) S. Naito, M. Hirai: Yakugaku Kenkyu, **36**, 163 (1965).
6) S. Naito, C. Nakahara: *Ibid.*, **36**, 168 (1965).
7) S. Naito: Yakugaku Kenkyu, **35**, 354 (1964).
8) S. Naito, Y. Katayama, K. Ekuni: Yakuzaigaku, **26**, 33 (1966).
9) S. Naito: Yakugaku Kenkyu, **35**, 384 (1964).
10) S. Naito, A. Awataguchi, A. Kise: Yakuzaigaku, **26**, 37 (1966).
11) S. Naito, M. Hayashi: Kyoto Yakka Daigaku Gakuho, **12**, 21 (1964).
12) M. Nakagaki, N. Koga, H. Terada: Yakugaku Zasshi, **83**, 586 (1963).

Table I. Binding of GGE with Serum Protein following Oral Administration 500 mg./kg. of GGE to Rabbit

I-1 1 Hour after Dose

Rabbit No.	Ca	Cb buffer. ml. (inside) 2.0	1.0	0.5	0
13	194	136 (29.9)			
14	752	626 (16.8)			
15	332	213 (35.8)			
16	296	224 (24.3)			
17	364	295 (19.0)			
Mean Value	388	299 (25.2)			
18	420		385 (8.4)	360 (14.3)	315 (25.0)
19	560		380 (30.0)	350 (37.5)	450 (19.8)
20	365		230 (34.2)	220 (39.5)	255 (30.1)
21	550		418 (24.0)	430 (21.8)	446 (18.9)
Mean Value	474		353 (24.2)	340 (28.3)	367 (23.5)

I-2 2 Hours after Dose

Rabbit No.	Ca	Cb buffer. ml. (inside) 2.0	1.0	0.5	0
18	280		258 (7.9)	226 (19.3)	148 (47.1)
19	410		305 (25.6)	315 (23.2)	335 (18.3)
20	210		125 (40.5)	113 (46.2)	120 (42.9)
21	465		400 (13.9)	432 (7.1)	390 (16.1)
Mean Value	341		272 (22.0)	272 (24.0)	248 (31.1)

The values in the parentheses show the binding ratios of GGE with serum protein
Ca : serum level of GGE (mcg./ml.)
Cb : concn. of GGE (mcg./ml.) after equilibrium dialysis (outside of bag)
Experimental Conditions :
rabbit serum 1.0 ml. in the inside of bag.
eight milliliters of the phosphate buffer soln.(pH 7.4) in the outside of bag.

Table II. Binding of GGE with Serum Protein of Rabbit and Human (*in vitro*)

	Ca	Cb	Cc	Cd
Rabbit	1.44	a)	4.62×10^{-5}	d)
		b)	3.43×10^{-4}	e)
		c)	6.74×10^{-4}	f)
	2.88	a)	7.54×10^{-5}	d)
		b)	3.40×10^{-4}	e)
		c)	6.78×10^{-4}	f)
Human	1.48	a)	6.33×10^{-5}	d)
		b)	2.74×10^{-4}	e)
		c)	6.70×10^{-4}	f)
	2.96	a)	6.13×10^{-5}	d)
		b)	3.32×10^{-4}	e)
		c)	6.44×10^{-4}	f)

Ca : Concn. (%) of serum protein in 3 ml. phosphate buffer soln. (pH 7.4) in the inside of bag.
Cb : Initial concn. (*M*/L.) of GGE in 8 ml. phosphate buffer soln. (pH 7.4) in the outside of bag.
Cc : Concn. (*M*/L.) of GGE in the outside of bag soln. after equilibrium dialysis.
Cd : Concn. (*M*/L.) of GGE in the outside of bag soln. after equilibrium dialysis, when the inside of bag soln. is phosphate buffer soln. (pH 7.4) without protein.
a) 1×10^{-4} b) 5×10^{-4} c) 1×10^{-3} d) 6.50×10^{-5} e) 3.27×10^{-4} f) 6.74×10^{-4}

Result and Discussion

Plasma data from GGE are shown in Table Ⅲ and Fig. 2. The highest plasma concentration was obtained at half an hour to one hour, similarly to the report of Morgan, *et al.*[3] This drug seems to be absorbed by passive transport, since the peak of blood level is almost proportional to the dose.

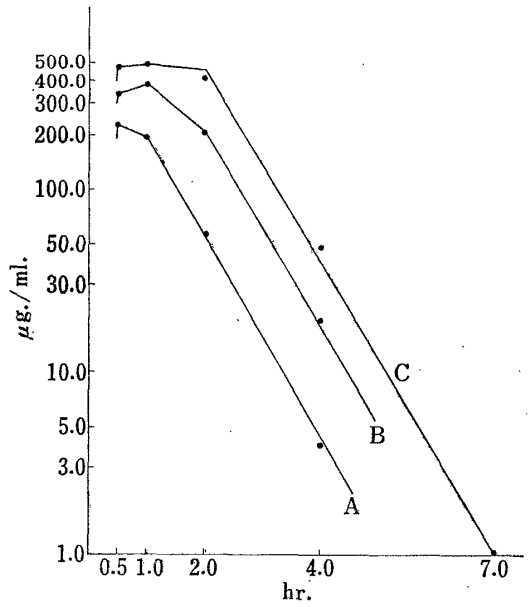

Fig. 2. Mean Plasma Level of GGE after Oral Administration to Rabbits at Different Doses

A 250 mg./kg. of GGE
B 375 mg./kg. of GGE
C 500 mg./kg. of GGE

The binding of GGE with rabbit serum proteins *in vivo* at different concentrations of blood level are shown in Table I. It was confirmed that the binding-ratios of GGE with serum proteins (in rabbit) are varied, followed the experimental conditions in dialysis. As the results, the averaged binding ratios was about 25.3% and 25.7% at 1 and 2 hour, respectively, after ingestion of the drug (500 mg./kg. in rabbit). The binding of GGE with human and rabbit serum proteins *in vitro* are shown in Table Ⅱ. It may be said positively that there is no distinct combination between GGE and serum protein unlikely to *in vivo*.

As reported by some workers,[13~17] Mephenesin, one of the similar compounds to GGE, undergoes in animals a progressive oxidation of

Table Ⅲ. Plasma Level of GGE in μg./ml. following Oral Administration to Rabbits

Rabbit No.	Body weight (kg.)	Dose (/kg.)	Hours after Dose 0.5	1.0	2.0	4.0	7.0
1	2.0	500 mg.	435	492	318	0	0
2	1.9		504	504	405	56	0
3	1.9		435	420	330	69	0
4	2.0		540	540	615	67	4
Mean Value	2.0		479	489	417	48	1
5	2.0	375 mg.	305	315	73	0	0
6	2.1		360	510	430	40	0
7	2.0		350	345	215	25	0
8	1.9		350	360	100	10	0
Mean Value	2.0		341	383	205	19	0
9	2.1	250 mg.	240	215	95	0	0
10	2.1		188	175	25	0	0
11	2.2		241	187	25	0	0
12	2.0		250	205	72	15	0
Mean Value	2.1		230	195	57	4	0

13) J. B. Wyngaarden, L. A. Woods, M. H. Seevers: Proc. Soc. Exp. Biol. and Med., **66**, 256 (1947).
14) E. L. Graves, T. J. Elliott, W. Bradley: Nature, Lond., **162**, 257 (1948).
15) R. F. Riley, F. M. Berger: Arch. Biochem., **20**, 159 (1949).
16) R. F. Riley: J. Am. Chem. Soc., **72**, 5712 (1950).
17) *Idem*: J. Pharmacol. and Exper. Therap., **99**, 329 (1950).

the glycerol chain, giving rise to α-hydroxy-α-(*o*-toloxy)propionic acid and, to a much lesser extent to *o*-toloxyacetic acid. The analogous metabolites are, hence, expected to isolated from GGE. Results of further studies of urinary excretion and metabolites of GGE will be reported.

[Chem. Pharm. Bull. 15(9)1426~1428(1967)]

UDC 547.387.04 : 547.835.04

Tetsuzo Kato and Yutaka Yamamoto*1 : Studies on Ketene and Its Derivatives. XVIII.*2 Reaction of Diketene with Acridine.

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(Received December 3, 1966)

In the previous papers[1] of this series, we have reported reaction of diketene with the C=N double bond in the aromatic heterocycles such as quinoline or phenathridine to give the Wollenberg's type compound (I).[2] Also, we have reported that diketene reacted with the C=N double bond of the Schiff base to give the alkylidene acetoacetamide (II).[3] The continuous study was undertaken to see if diketene could react with the C=N double bond of acridine in a similar fashion as described above. In the present paper we wish to report reaction of diketene with acridine proceeded in a different manner from the case of quinoline or phenanthridine, affording 9-substituted acridan.

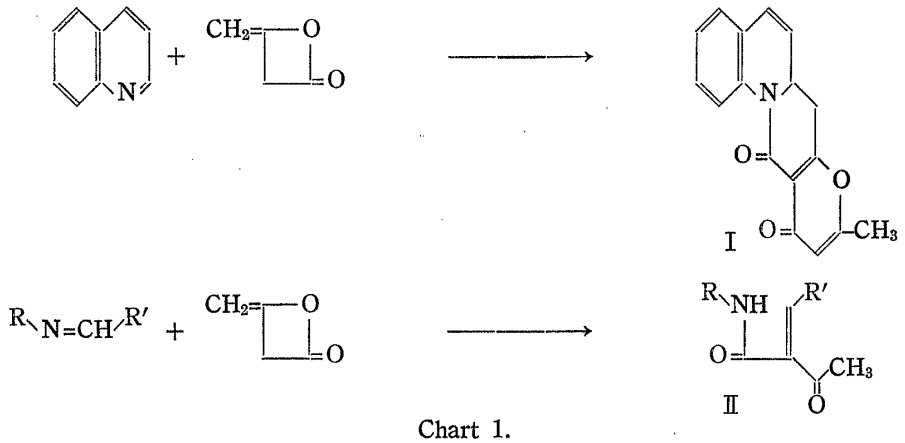

Chart 1.

When acridine was treated with an excess of diketene, colorless needles of m.p. 140~141°, $C_{16}H_{15}ON$ (III), were obtained. The ultraviolet spectrum of this compound showed only one maximum absorption at 281 mμ, on the other hand, in the case of acridine or 9-acridylacetone (IV) two maximum peaks appeared at 249, 357 mμ and 252, 360 mμ, respectively. The infrared spectrum of III in chloroform exhibited a characteristic peak at 1710 cm^{-1} (C=O) and the NH absorption band at 3448 cm^{-1}. In the nuclear magnetic resonance (NMR) spectrum signals of methyl protons (3H, 1.83 p.p.m., singlet),

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*2 Part XVII. T. Kato, Y. Yamamoto: This Bulletin, **15**, 1334 (1967).

1) T. Kato, T. Kitagawa, Y. Yamamoto: Yakugaku Zasshi, **83**, 267 (1963); T. Kato, T. Kitagawa: *Ibid.*, **84**, 874 (1964); T. Kato, Y. Yamamoto: This Bulletin, **14**, 752 (1966).

2) O. Wollenberg: Ber., **67**, 1675 (1934); J. Berson, W. Jones: J. Am. Chem. Soc., **78**, 1625 (1956).

3) T. Kato, Y. Yamamoto: This Bulletin, **13**, 959 (1965); *Idem*: Yakugaku Zasshi, **87**, 691 (1967).