ribed in this report that the effect should be caused by the inhibition of the process for the viral reproduction, and its action site should be in intracellular site. The study as to the mode of action of the inhibitory effect of guanidine nitrate is now going on. The results of this work will be reported in future. At any rate, to find highly effective antiviral agants, the findings described in this report concerning the effect of guanidine should be a milestone. Thus, the research regarding the relationship between the antiviral property and the chemical structure of guanido group containing compound is going on, too. These results also will be described in another paper.

Summary

The inhibitory effect of guanidine nitrate on the growth of several small animal viruses was investigated. It was found that guanidine inhibited the growth of both poliomyelitis and measles viruses. The findings presented in this report suggest that guanidine inhibits the intracellular multiplication of these viruses and block the replication mechanism of the viruses.

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3. Hisao Tsukamoto, Hidetoshi Yoshimura, and Hiroyuki Ide: Metabolism of Drugs. XXXII.[*1] The Metabolic Fate of Secobarbital [5-Allyl-5-(1-methylbutyl)barbituric Acid].

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In the previous paper[1] the metabolic fate of thiamylal, thio-analogue of secobarbital [5-allyl-5-(1-methylbutyl)barbituric acid] was reported and a caboxylic acid derivative was isolated and characterized as a major metabolite. However further studies on this metabolic fate seemed to be rather difficult, because the paper chromatogram of the urine extract of rabbits to which this drug was administered was so complicated that the resulted product might be of simultaneous formation of desulfuration and oxidation reaction. However, a comparison of paper chromatograms of urine extracts treated by both Thiamylal and secobarbital suggested that some of the metabolites of the former could be identical with that of the latter.

It has therefore been undertaken to elucidate firstly the metabolic fate of Secobarbital which has not been known so far.

Secobarbital [5-allyl-5-(1-methylbutyl)barbituric acid] is one of the common sedatives and hypnotics of short duration. It seems to undergo similar oxidation processes and lose its activity in animal body as the other short-acting barbiturates do.[2] It possesses however allyl and 1-methylbutyl side chains, and so it is very interesting to know which is more susceptible to biological oxidation.

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*1 Part XXXI. S. Toki, K. Toki, H. Tsukamoto: This Bulletin, **10**, 708 (1962).
*2 Katakasu, Fukuoka (塚元久雄, 吉村英敏, 井出博之).
1) H. Tsukamoto, H. Ide, E. Takabatake: This Bulletin, 8, 236 (1960).
2) J. Raventos: J. Pharm. and Pharmacol., **6**, 217 (1954); B.B. Brodie: *Ibid.*, **8**, 1 (1956).

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It is now shown in this paper that two metabolites, in addition to the secobarbital recovered were isolated from the rabbits urine administered secobarbital and their structures were established as 5-allyl-5-(1-methyl-3-hydroxybutyl)- and 5-allyl-5-(1-methyl-3-carboxypropyl)barbituric acid, respectively. A metabolite with decomposed barbituric acid ring was also detected by the paper chromatography, but among the metabolites isolated, allyl side chain was remained unchanged.

Methods and Results

Drug Administration and Urine Extraction——Secobarbital*[3] in the doses of 50 mg./kg. body wt. was administered as a freshly prepared aq. solution containing 1.1 equiv. NaOH by a stomach tube to rabbits weighing about 2.5 kg. and the 24 hr. urine was collected.*[4] A total of secobarbital administered was about 2.2 g. (18 rabbits) and the volume of collected urine was 4.5 L. The urine was filtered through the cotton, adjusted to pH 4 with conc. HCl and extracted continuously with AcOEt. A reddish-brown oily substance (10.5 g.), remained after an evaporation of the solvent, was dissolved again in 100 cc. of AcOEt and filtered off insoluble matters (mainely urea). A clear AcOEt solution was shaken with dil. NaOH at pH 10~11. The aq. alkaline phase was once washed with AcOEt, made acidic with conc. HCl, and extracted with AcOEt. This extract was shaken with satd. $NaHCO_3$ solution and the separated AcOEt phase was evaporated to dryness, affording 1.5 g. of syrup (SA-fraction).

The aq. $NaHCO_3$ phase was adjusted to pH 2 with conc. HCl and extracted with AcOEt. After evaporating the solvent, 0.8 g. of oily substance was remained (SB-fraction).

Paper Chromatography——Each fraction (SA and SB-fractions) was dissolved in MeOH and developed by ascending chromatography on a filter paper (Toyo Roshi No. 50) using the solvent system of BuOH-EtOH-conc. NH_4OH (4:2:1.2). The following reagents were used for displaying the chromatogram same as in the previous paper.[1)] a) (Mn) : 1% $KMnO_4$ b) (Hg) : satd. $HgNO_3$ c) (Co) : $Co(NO_3)_2$ and NH_3 damp. d) (UV) : UV-lamp.*[5] As shown in Table I, SA-fraction included at least three metabolites, spot 2, 3, and 4, and their Rf values were identical with hydroxy-secobarbital, and unchanged secobarbital, and ring-destroyed metabolite, respectively. Spot 1 appeared only in SB-fraction and this structure was established as a secobarbital carboxylic acid. On these structure, there will be discussed later.

Table I. Paper Chromatogram of Urine Extract

Spot No.	Rf	Reagents				Fractions		Identification
		Mn	Hg	Go	UV	SA	SB	
1	0.14~0.15	+	+	+	+	−	+	secobarbital-COOH
2	0.62~0.63	+	+	+	+	+	−	secobarbital-OH
3	0.72~0.73	+	+	+	+	+	−	unchanged secobarbital
4	0.83~0.85	+	−	−	−	+	−	ring-destroyed metabolite

Isolation, Characterization, and Identification of Metabolites : 1) SA-fraction——This (1.5 g.) was dissolved in benzene and chromatographed through an alumina column (30 g. of Al_2O_3), in which fractions were separated by a stepwise elution using the solvent of benzene, Me_2CO, and finally MeOH. Metabolite 4 (ring-destroyed metabolite) was eluted with benzene, which was failed to crystallize and proved only by paper chromatography. It did not appear, of course, in a control rabbits urine extract and did not show any color reaction by barbiturate but indicated a possession of an unsaturation of allyl group with (Mn)-reagent. The Rf value was also quite reasonable for the acetylurea derivative, but no further studies on this metabolite was undertaken.

Barbiturates were detected in the following two fractions, benzene-Me_2CO (99:1) and Me_2CO-MeOH (4:1) effluents by the paper chromatographical examination of the effluents. The former fraction was recrystallized from 30% aq. EtOH to colcrless crystals, m.p. 98~99° (8 mg.), which was identified with unchanged secobarbital by the mixed m.p. test,paper chromatography and IR spectrum. The latter fraction was further purified by dissolving in *N* NaOH, reacidification, extraction with AcOEt and treatment with charcoal. The yielded crude crystallized compound, m.p. 148~158°, was recrystallized from AcOEt to 65 mg. of colorless crystals, m.p. 167°. $[\alpha]_D^{15}+19.3°$ (metabolite 2). The elemental analysis agreed with

*[3] Kindly donated by the Yoshitomi Pharmaceutical Co., Ltd.

*[4] A preliminary test showed that the excretion of metabolites ended practically within 24 hr. after medication.

*[5] Manasulu Light (Manasulu Chem. Ind, Co., Ltd.) was used.

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a hydroxy-secobarbital. *Anal* Calcd. for $C_{12}H_{18}N_2O_4$: C, 56.71; H, 7.07; N, 11.01. Found : C, 56. 70; H, 7.53; N, 10.87. UV ($\lambda_{max}^{Borate\ buffer\ pH\ 10}$ 240 mμ) and IR : λ_{max}^{Nujol} 2.75 (ν_{OH}); 3.10, 3.22 (ν_{NH}); 5.66, 5.75, 5.88 ($\nu_{C=O}$); 6.05 ($\nu_{C=C}$); 9.95, 10.86 μ ($\delta_{CH=CH_2}$), indicated also that this metabolite should be a hydroxy-secobarbital, in which one of the alkyl side chain would be oxidized to the hydroxy alkyl group. The position of this hydroxy group was finally concluded from the fact that this metabolite produced crystalline CHI_3, m.p. 118° by heating with 3*N* NaOH and I_2-KI solution. The structure of this metabolite should therefore be 5-allyl-5-(1-methyl-3-hydroxybutyl)barbituric acid.

2) **SB-fraction**——This fraction (0.8 g.) was dissolved in AcOEt and extracted with satd. $NaHCO_3$ solution. The $NaHCO_3$ layer was adjusted to pH 7 with dil. HCl, filtered off the separated oily substances, and then the filtrate was further acidified to pH 2 with conc. HCl. The solution was extracted with AcOEt, dried over Na_2SO_4, and evaporated to dryness, to this residue. (0.3 g.), a few drops of 30% aq. EtOH was added and kept in a refrigerator for several days. The solid deposited was recrystallized from AcOEt to colorless crystals, m.p. 197° (metabolite 1). Yield was 140 mg. *Anal*. Calcd. for $C_{12}H_{16}O_5N_2$: C, 53.75; H, 5.96; N, 10.44. Found : C, 53.94; H, 6.04; N, 10.10. UV : $\lambda_{max}^{Borate\ buffer\ pH\ 10}$ 240 mμ. IR: λ_{max}^{Nujol} 3.10, 3.22 (ν_{NH}); 5.70~5.85 ($\nu_{C=O}$); 6.08 ($\nu_{C=C}$); 9.95, 10.85 μ ($\delta_{CH=CH_2}$). The analytical values and the spectral data which showed the intact existence of barbituric acid ring and allyl sidechain, indicated that it should be secobarbital carboxylic acid, in which one of the terminal methyls of 1-methylbutyl side chain should be oxidized to the carboxyl group. This assumption was exactly confirme by the synthesis of 5-propyl-5-(1-methyl-3-carboxypropyl) barbituric acid and its identity with the catalytic reduction product of metabolite 1 as shown below.

3) **Reduction of Metabolite 1**——A solution of 158 mg. of the metabolite in 50% aq. EtOH was hydrogenated catalytically with 60 mg. of PtO_2. After one molar equiv. of H_2 was absorbed (16 min.), it was treated as usual and 140 mg. of crystals, m.p. 182° were obtained after recrystallization from AcOEt. *Anal*. Calcd. for $C_{12}H_{18}N_2O_5$: C, 53.35; H, 6.66; N, 10.36. Found : C, 53.44; H, 6.48; N, 10.21. The IR absorption bands of the allyl group disappeared completely in the spectrum of this dihydro derivative, which indicated that metabolite 1 possessed an intact allyl group in it.

Synthesis of 5-propyl-5-(1-methyl-3-carboxypropyl)barbituric Acid——It was performed according to an essentially analogous method with the synthesis of 5-ethyl-5-(1-methyl-3-carboxypropyl)barbituric acid by H. B. Wood. Jr. and E. C. Horing,[3] as shown in Chart 2.

1) **Diethyl (1-methyl-4-pentenyl)propylmalonate**——To EtONa prepared from 3.5 g. of Na, 50 ml. of redistilled diethyl carbonate and 48.5 g. of diethyl propyl-malonate were added. The mixture was distilled under reduced pressure until EtOH was removed, and 21.5 g. of 2-bromo-5-hexene was added dropwise. The residue was treated with 180 ml. of water and extracted with $(iso-Pr)_2O$. The extracts were combined, washed with satd. NaCl solution, dried, and distilled *in vacuo*. The yield of the product was 16.0 g. IR : λ_{max}^{Liquid} 5.68~5.78 ($\nu_{C=O}$); 6.05 ($\nu_{C=C}$); 10.05, 10.95 μ ($\delta_{CH=CH_2}$).

2) **5-Propyl-5-(1-methyl-4-pentenyl)barbituric Acid**——To a solution of EtONa prepared from 2.8 g. of Na and 100 ml. of anhyd. EtOH, were added 7.3 g. of dry, finely ground urea and 10 g. of diethyl-(1-methyl-4-pentenyl)propylmalonate. After the mixture was heated under reflux for 12 hr., EtOH was removed by evaporation under reduced pressure using a bath maintained at 50°. The residue was dissolved in 100 ml. of ice H_2O, and extracted with Et_2O. Solution was then acidified with 6*N* H_2SO_4, extracted with Et_2O, and evaporated. 3.8 g. of oily substance was obtained showing

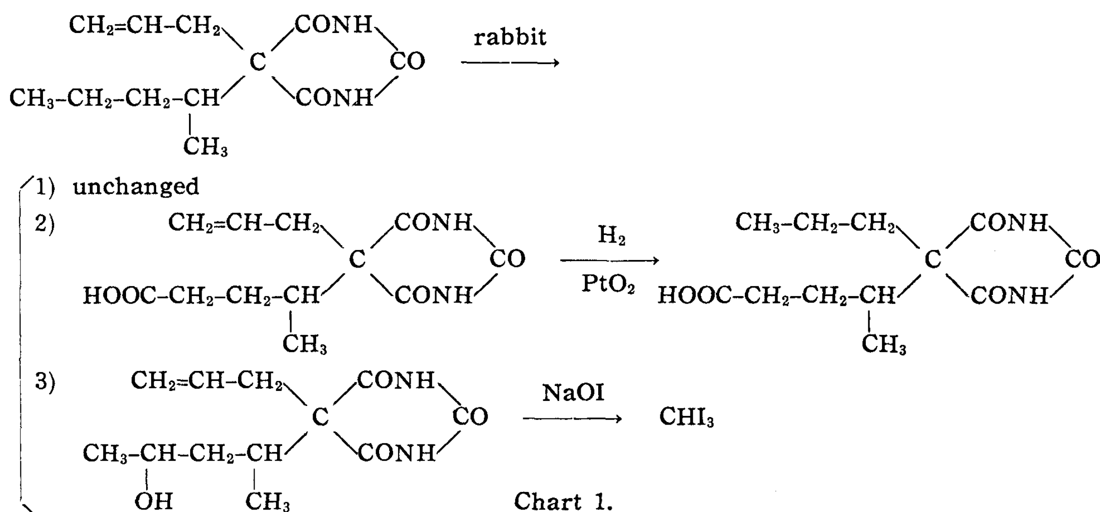

Chart 1.

3) H. B. Wood. Jr., E. C. Horing : J. Am. Chem. Soc., **75**, 5511 (1953).

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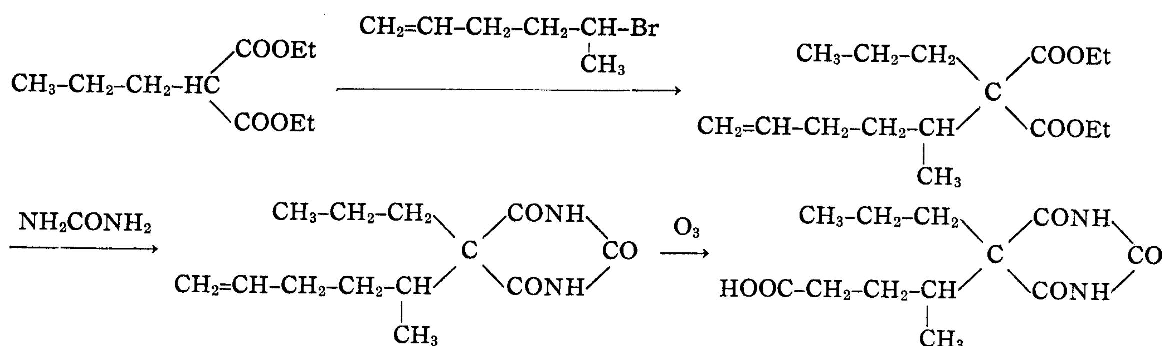

Chart 2.

characteristic UV and IR spectra of barbiturates, and indicated a reasonable purity in paper chromatogram, but did not crystallize in spite of various attempts. UV : $\lambda_{max}^{Borate\ buffer\ pH\ 10}$ 224 mμ. IR : λ_{max}^{Nujol} 3.05, 3.16 (ν_{NH}); 5.70~5.90 ($\nu_{C=O}$); 10.05, 10.94 μ ($\delta_{CH=CH_2}$).

3) **5-Propyl-5-(1-methyl-3-carboxypropyl)barbituric Acid**——A solution of 1 g. of 5-propyl-5-(1-methyl-4-pentenyl)barbituric acid in 100 ml. of methylene chloride was cooled to 0° and subjected to a stream of ozonized oxygen (0.001 mole of ozone per min.) for 6 hr. after addition of 200 ml. of H_2O and 5 ml. of 30% H_2O_2 to the reaction mixture, and the evaporation of methylene chloride, the aq. solution was extracted with Et_2O, which was shaken with satd. $NaHCO_3$ solution. The aq. $NaHCO_3$ layer was separated, acidified to pH 2 with conc. HCl, and extracted with AcOEt. The solid material obtained after evaporation of AcOEt was recrystallized from AcOEt to colorless crystals, m.p. 180~181°. (60 mg.). This product was identical with dihydro derivative of metabolite, m.p. 182° by the mixed m.p. test, UV and IR spectra, and paper chromatography.

Discussion

There is an interesting metabolic difference between the major pathways of pentobarbital and its thio-analogue, Thiopental. The former is metabolized mainly to (ω-1)-hydroxy compound,[4] while the latter to ω-carboxylic acid derivative.[3,5] Considering the previous results that ω-carboxylic acid was the main metabolite of Thiamylal,[1] it was assumed that the main metabolite of secobarbital might be (ω-1)-hydroxy derivative.

The result obtained here showed unexpectedly that it was not only (ω-1)-hydroxy- but also ω-carboxylic acid derivatives and further, the latter was isolated twice as much as the former, although the comparison of the amounts was not followed by quantitative experiment.

It is however important to note that the amount of hydroxy metabolite excreted consists of a minor part in Thiamylal treated rabbits,[1] but major in secobarbital.

The next interest was focussed on the metabolic fate of allylic methylene in secobarbital which seemed also to be attacked by biological oxidation in addition to ω- and (ω-1) position of 1-methylbutyl side chain, but it was shown that the allyl group remained intact in all of the metabolites isolated in the present study. It does not seem, however, to be so unreasonable, because the location of the allylic methylene is just adjacent to *tert* carbon atom in barbituric acid and therefore it probably hindered sterically. This presumption is considered to be rather coincident with our previous findings,[6,7] that cyclohexenyl side chain in methylhexabital and ethylhexabital was oxidized only at one of their two allylic position, while the others did not suscept a biological attack probably due to the same reason as in secobarbital.

4) E.W. Maynert, J.M. Dawson : J. Biol. Chem., **159**, 389 (1952).
5) B.B. Brodie, *et al.* : J. Pharmacol. Exptl. Therap., **98**, 85 (1950).
6) H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, **3**, 239 (1955).
7) *Idem* : *Ibid.*, **4**, 368 (1955); H. Yoshimura : *Ibid.*, **5**, 561 (1957).

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It was reported by Maynert and Dawson,[4] that two diasteroisomeric alcohols were isolated as a main metabolite of pentobarbital, but in the case of secobarbital only one of possible diastereoisomeric alcohols was isolated.

Although there must be certain conjugation forms as metabolites, the search for the free forms were only performed in the present study.

As discussed in the previous paper,[1] many metabolites were produced in rabbits administered by Thiamylal, some of which were presumed to be identical with secobarbital and its metabolites by paper chromatography. The studies on the metabolic fate of Thiamylal are continued in the hope of being able to confirm these assumption by utilizing the present study.

The authors are deeply grateful to Mrs. S. Matsuba for the elemental analysis, to Messrs. H. Matsui and K. Hikida for the determination of infrared spectra, and also to the Yoshitomi Pharmaceutical Co. Ltd., for their supply of secobarbital.

Summary

The metabolic fate of secobarbital [5-allyl-5-(1-methylbutyl)barbituric acid] in rabbits were studied and two main metabolites were isolated in addition to the unchanged secobarbital.

The structure of the metabolites were established as 5-allyl-5-(1-methyl-3-carboxypropyl)- and 5-allyl-5-(1-methyl-3-hydroxybutyl)barbituric acid.

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4. Hisashi Nogami,*1 Shoji Awazu,*1 and Yoshio Kanakubo*2 :

Studies on Decomposition and Stabilization of Drugs in Solution. XIII. On Sodium Lauryl Sulfate.*3

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The authors[1,2] have been investigating the stabilization of drugs by surface active agents (surfactants), and it was found, in part XII[3], that the hydrolysis of methantheline bromide in a presence of H^+ was accelerated by sodium lauryl sulfate (SLS), and it was ascribed to the attraction of H^+ on the environment of SLS micelle. If this assumption would be correct, the hydrolysis of an ionic surfactant might be influenced by the appearance of micelle as the simpler case than that of methantheline bromide,[3] and the rate of hydrolysis should vary near its critical micelle concentration (CMC). As SLS has been used in this project, it was selected as the first object, and its stability at various pH as well as concentration of SLS was studied.

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*3 Presented at the Kanto Local Meeting of the Pharmaceutical Society of Japan, Tokyo, June, 1961.
1) H. Nogami, *et al.* : This Bulletin, 8, 1136 (1960).
2) *Idem* : *Ibid.*, **10**, 503 (1962).
3) *Idem* : *Ibid.*, **10**, 1158 (1962).

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