

**6-Deoxy-6-mercapto-1,2-O-isopropylidene-3,5-benzylidene- $\alpha$ -D-glucofuranose (XV)**—A solution of 3.6 g. of (XIII) in 20 cc. of MeOH containing 0.23 g. of Na (1 mol. equiv) was left to stand at room temperature for 6 hr. Then the mixture was neutralized with AcOH and 3.2 g. of colorless crystals were obtained. Recrystallization from EtOH gave (XV), m.p. 129°;  $[\alpha]_D^{20} + 16.8$  (c=1.0, CHCl<sub>3</sub>). (XV) gave color reaction with sodium nitroferricyanide and was not hygroscopic. *Anal.* Calcd. for C<sub>16</sub>H<sub>20</sub>O<sub>5</sub>S: C, 59.25; H, 6.22; S, 9.87. Found: C, 59.04; H, 6.39; S, 9.90.

**6-Deoxy-6-mercapto-6-S-methyl-1,2-O-isopropylidene-3,5-benzylidene- $\alpha$ -D-glucofuranose (XIV)**—To a solution of 2.5 g. of (XIII) in 25 cc. of Me<sub>2</sub>CO, 8 cc. of Me<sub>2</sub>SO<sub>4</sub> and 18 cc. of 30% NaOH were added alternately at 50° with stirring for 1.5 hr. After heating at 70° for 15 min., the mixture was poured into 200 cc. of H<sub>2</sub>O and neutralized with 50% AcOH. The precipitates were recrystallized to colorless crystals from EtOH, m.p. 124~126°;  $[\alpha]_D^{20} + 6$  (c=1.5, CHCl<sub>3</sub>). (XIV) was also obtained from (XV) with CH<sub>2</sub>N<sub>2</sub> in Et<sub>2</sub>O in the usual manner. *Anal.* Calcd. for C<sub>17</sub>H<sub>22</sub>O<sub>5</sub>S: C, 60.34; H, 6.55; S, 9.46. Found: C, 60.60; H, 6.72; S, 9.29.

**Oxidation of (XV) by iodine to disulfide (XVI)**—To a solution of 1.0 g. of (XV) or (XIII) in 20 cc. of 0.5N NaOH-EtOH the calculated amount of I<sub>2</sub> dissolved in EtOH was added. After 2 hr., the precipitates were filtered off and washed with H<sub>2</sub>O and EtOH. Recrystallization from CHCl<sub>3</sub>-EtOH gave white crystalline powder, m.p. 223°;  $[\alpha]_D^{20} + 3$  (c=1.3, CHCl<sub>3</sub>). *Anal.* Calcd. for C<sub>22</sub>H<sub>38</sub>O<sub>10</sub>S<sub>2</sub>: C, 59.12; H, 5.88; S, 9.62. Found: C, 59.26; H, 6.00; S, 9.54.

**Desulfurization of (XIII) and (XIV) by Raney-Ni**—From (XIII) or (XIV) 6-deoxy-1,2-isopropylidene-3,5-benzylidene- $\alpha$ -D-glucofuranose (XVII), m.p. 111°<sup>9)</sup> was obtained in 60~70% yield in the same manner as described for (VIII).

### Summary

6-Deoxy-6-thio-D-glucose was prepared by hydrolysis of 6-deoxy-6-mercapto-6-S-acetyl-1,2,3,4-tetra-O-acetyl- $\beta$ -D-glucopyranose and 6-deoxy-6-mercapto-6-S-acetyl-1,2-O-isopropylidene-3,5-benzylidene- $\alpha$ -D-glucofuranose which were synthesized from corresponding 6-*p*-toluenesulfonyl derivatives and potassium thioacetone in boiling acetone.

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### 92. Minoru Yoshimura<sup>\*2</sup> and Hisao Tsukamoto<sup>\*3</sup>: Metabolism of Drugs. XXX.\*<sup>1</sup> The Biotransformation of Drugs having Cyclohexene Ring. (3). The Synthesis and Metabolism of 5-Ethyl-5-(1-cyclohexenyl)-4,6-dioxohexahydropyrimidine.

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It was shown in the previous paper on the metabolism of drugs having a cyclohexene ring that 5-ethyl-5-(1-cyclohexenyl)barbituric acid (EHB),<sup>1,2)</sup> 1,5-dimethyl-5-(1-cyclohexenyl)barbituric acid (MHB),<sup>3)</sup> and 2-alkyl- or 2-phenyl-2-(1-cyclohexenyl)glutaramide<sup>4)</sup> underwent oxidation *in vivo* or *in vitro* at 3-position of the cyclohexene ring to yield oxo and hydroxyl derivatives. These drugs have a common moiety of -CO-NH-

\*<sup>1</sup> Part XXIX: This Bulletin, 10, 540(1962).

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1) H. Tsukamoto, E. Takabatake, H. Yoshimura: This Bulletin, 2, 201 (1954).

2) H. Tsukamoto, H. Yoshimura, S. Toki: *Ibid.*, 3, 239 (1955).

3) H. Tsukamoto, H. Yoshimura: *Ibid.*, 3, 364 (1955).

4) *Idem*: *Ibid.*, 9, 584 (1961).

CO- and exhibit hypnotic activity. The reduction of 2-position of the barbituric acid portion results in the increase of anticonvulsant activity as seen in primidon (5-ethyl-5-phenyl-4,6-dioxohexahydropyrimidine) and phenobarbital (5-ethyl-5-phenyl-barbituric acid), and the replacement of the phenyl group by a cyclohexene ring decreases the duration of the activity, because it causes rapid metabolism of the drugs in the liver. From these viewpoints, it seems interesting to study the pharmacological activity and metabolism of cyclohexenyl substituted 4,6-dioxohexahydropyrimidines. It is the purpose of this paper to describe the synthesis and the *in vivo* metabolism of 5-ethyl-5-(1-cyclohexenyl)-4,6-dioxohexahydropyridine. This compound can be obtained by the reduction of 2-position of EHB or by the replacement of the phenyl group of primidon by a cyclohexene ring.

### Experimentals\*<sup>4</sup>

**Synthesis of 5-Ethyl-5-(1-cyclohexenyl)-4,6-dioxo-hexahydropyrimidine (ECP)**—This compound was prepared by the hydrogenolysis of the corresponding 2-thiobarbituric acid. To a suspension of about 60 g. of Raney-Ni catalyst\*<sup>5</sup> in 600 cc. of 95% EtOH and 70~100 cc. of H<sub>2</sub>O, 15 g. of the powdered 5-ethyl-5-(1-cyclohexenyl)-2-thiobarbituric acid\*<sup>6</sup> was added and the mixture was refluxed for 4~5 hr. After cool, the solvent was decanted and the Ni was washed with hot EtOH. The combined EtOH solutions were concentrated at 40~50° *in vacuo*. The solid deposited on cooling was recrystallized from EtOH to crystals of m.p. 269° (decomp.). Yield, 11.5 g. (90.6%). *Anal.* Calcd. for C<sub>12</sub>H<sub>18</sub>O<sub>2</sub>N<sub>2</sub>: C, 64.84; H, 8.16; N, 12.60. Found: C, 64.93; H, 8.20; N, 12.57.

**Oxidation of ECP with Chromium Trioxide**—To a well stirred suspension of 5 g. of powdered ECP in Ac<sub>2</sub>O, a solution of 5.6 g. of CrO<sub>3</sub> in 40 cc. of Ac<sub>2</sub>O was added at 10~15° during 1 hr., the reaction mixture was kept at about 35° for an additional 1 hr. with stirring, and allowed to stand for 2 days. The solvent was distilled off *in vacuo* and 100 cc. of H<sub>2</sub>O was added to the residue. The aqueous solution was repeatedly extracted with AcOEt, and the combined extracts were washed with a small quantity of H<sub>2</sub>O and dried over anhyd. Na<sub>2</sub>SO<sub>4</sub>. The reddish brown oily residue obtained after the evaporation of solvent solidified upon scratching. This solid was repeatedly recrystallized from MeOH to colorless plates of m.p. 227~229° (decomp.). Yield, 28~30%. The melting point of these crystals was not depressed or admixture with 5-ethyl-5-(3-oxo-1-cyclohexenyl)barbituric acid (3-oxo EHB) prepared by the same oxidation of EHB as shown in the previous paper of this series,<sup>1)</sup> or by the oxidation of the corresponding 2-thiobarbituric acid with CrO<sub>3</sub>. *Anal.* Calcd. for C<sub>12</sub>H<sub>14</sub>O<sub>4</sub>N<sub>2</sub>: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.63; H, 5.82; N, 11.35.

**Drug Administration and Urine Extraction**—ECP in the doses of 300 mg./kg. body wt. was orally administered as 1% propylene glycol solution through a stomach tube to rabbits weighing 2.5~3.5 kg. which were fasted for 24 hr. After the medication, the food was given and the urine was then collected during 48 hr. into the bottles containing a few drops of toluene and 1 cc. of glacial AcOH to prevent alkaline oxidation of any metabolites excreted. In this case, some paralytic, but not hypnotic action was observed.

The collected urine was filtered through the cotton, brought to pH 5.0 with conc. H<sub>2</sub>SO<sub>4</sub>, and heated on a boiling water bath for 5 hr. to hydrolyze the conjugated metabolites. After cool, it was continuously extracted with Et<sub>2</sub>O for 35 hr. The reddish oily residue obtained after the evaporation of Et<sub>2</sub>O was kept in a vacuum desiccator containing CaCl<sub>2</sub> overnight and dissolved in Me<sub>2</sub>CO. The insoluble material was crystallized from MeOH or EtOH to give unchanged ECP as colorless plates of m.p. 267~269° (decomp.), undepressed on admixture with medicated ECP. *Anal.* Calcd. for C<sub>12</sub>H<sub>18</sub>O<sub>2</sub>N<sub>2</sub>: C, 64.84; H, 8.16; N, 12.60. Found: C, 65.32; H, 8.15; N, 12.67.

After removal of unchanged ECP, the Me<sub>2</sub>CO solution was passed through an alumina column and the eluate with MeOH gave a residue which crystallized from MeOH to colorless plates of m.p. 226~229° (decomp.), ECP-M(I). The melting point of this material was undepressed on admixture with 3-oxo-EHB (m.p. 227~229°), a CrO<sub>3</sub> oxidation product of ECP or EHB. *Anal.* Calcd. for C<sub>12</sub>H<sub>14</sub>O<sub>4</sub>N<sub>2</sub>: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.82; H, 5.48; N, 11.40.

\*<sup>4</sup> All melting points are uncorrected.

\*<sup>5</sup> The catalyst was prepared by the modified method of "Org. Synthesis"<sup>5)</sup>: After the addition of the alloy, the reaction mixture was finally heated at 80° for 1 hr. and allowed to stand for 1 or 2 days.

\*<sup>6</sup> This was synthesized by the usual procedure<sup>6)</sup> and melted at 188~189°.

5) R. Mazingo, D. E. Wolf, S. A. Harris, K. Folker: J. Am. Chem. Soc., **65**, 1013 (1943).

6) E. Volwiler, D. Taban: U. S. 2,153, 729 (C. A., **33**, 5599 (1939)).

The amounts of unchanged ECP and 3-oxo-EHB obtained from the urine of rabbits receiving ECP are shown in Table I.

TABLE I. Isolation of Unchanged ECP and 3-Oxo-EHB from the Urine of Rabbits Receiving ECP

No. of rabbit	Av. body wt. (g.)	Dose (mg./kg.)	Total (mg.)	Vol. of urine (cc.)	Unchanged ECP		3-Oxo-EHB	
					mg.	%	mg.	%
3	2700	300	2430	760	280	11.5	63	2.6
2	2850	300	1850	396	270	14.6	60	3.2

**Paper Chromatography of Urine Extract**—Samples dissolved in MeOH were chromatographed on a filter paper (Toyo Roshi, No. 50, 40×40 cm.) using the solvent system of iso-PrOH-CHCl<sub>3</sub>-conc. NH<sub>4</sub>OH (9:9:2, v/v) by the ascending technique. The solvent developed to a length of about 20 cm. during about 6 hr. at room temperature. The chromatographed paper was air-dried and sprayed with HIO<sub>4</sub>-KMnO<sub>4</sub> reagent<sup>7)</sup> to locate the unsaturated bond of cyclohexene ring.

The paperchromatograms of the Et<sub>2</sub>O extracts from both acidified urine and acidheat treated urine exhibited three spots of Rf 0.97, 0.23, and 0.03~0.04 as shown in Table II. Comparison with the paperchromatograms of authentic samples revealed that the spot at Rf 0.97 corresponds to unchanged ECP and that at Rf 0.23 to 3-oxo-EHB. The spot at Rf 0.03~0.04 is not yet identified. The crystals obtained from the urine extract were also identified in paper chromatography as unchanged ECP and 3-oxo-EHB.

TABLE II. Paperchromatography of Urine Extracts and Synthetic Samples

Et <sub>2</sub> O extract from			
acidified urine	0.97	0.23	0.04
acid-heat treated urine	0.97	0.23	0.03
Crystals obtained from urine			
unchanged ECP	0.97	—	—
ECP-M(I)	—	0.23	—
Synthetic materials			
ECP	0.97	—	—
CrO <sub>3</sub> oxidation product of ECP(3-oxo-EHB)	—	0.23	—
A mixture of ECP and 3-oxo-EHB	0.97	0.23	—

**Color Reaction**—The color reaction with Cu-pyridine reagent (a mixture of 10 cc. of 2% CuSO<sub>4</sub> and 2.5 cc. of pyridine) was blue in CEP and purple in ECP-M(I). The color reaction with 1% MeOH solution of Co(NO<sub>3</sub>)<sub>2</sub> and a trace of NH<sub>4</sub>OH was green in ECP and purple-red in ECP-M(I).

**Ultraviolet Absorption Spectra**—Data measured by a Shimadzu photoelectric spectrophotometer with standard 10 mm. square quartz absorption cells are shown in Table III.

TABLE III. Ultraviolet Absorption Spectra of ECP and ECP-M(I)

Sample	Solvent (10 γ/cc.)	Synthetic Sample		Extracted Sample	
		λ <sub>max</sub> (mμ)	log ε	λ <sub>max</sub> (mμ)	log ε
ECP	EtOH	218	3.26	217	3.27
	0.5N NaOH	225	3.73	224	3.46
ECP-M(I)	EtOH	223	3.24	225	3.21
	0.5N NaOH	223	3.52	223	3.53
		252	2.75	252	2.84
2,4-Dinitrophenylhydrazone <sup>a)</sup> of ECP-M(I)	EtOH	253	3.36	253	3.08
		372	4.18	372	4.45

a) This hydrazone melted with decomposition at 273~275° (from EtOH-AcOEt).

**Infrared Absorption Spectra**—The infrared absorption spectra of ECP, ECP-M(I), and its 2,4-dinitrophenylhydrazone measured by KBr pellet method in Koken DS. 301 are shown in Figs. 1~3. Both synthetic and extracted samples exhibited identical spectra.

7) H. Tsukamoto, H. Yoshimura, S. Toki: This Bulletin, 6, 15 (1958).

## Infrared Absorption Spectra

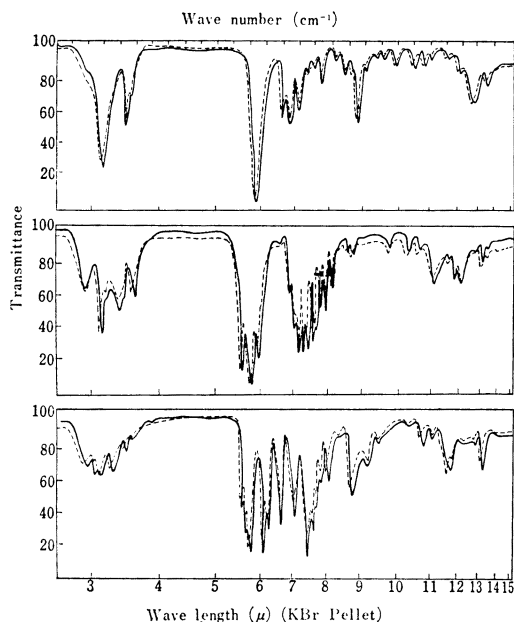


Fig. 1.

— ECP (Synthe.)  
 ..... Unchanged ECP  
 (fr. Urine)

Fig. 2.

— 3-Oxo-ECB (Synthe.)  
 ..... ECP-M(I)

Fig. 3.

— Hydrazone of 3-Oxo-ECB  
 ..... Hydrazone of ECP-M(I)

## Discussion

**On the Synthesis ECP**—It was reported by Boon, *et al.*<sup>8)</sup> that the hydrogenolysis of cyclohexenyl compounds using freshly prepared W2 or W5 Raney nickel catalyst gave cyclohexenyl compounds as main products. 5-Ethyl-5-(1-cyclohexenyl)-2-thiobarbituric acid was led by this method to 5-ethyl-5-cyclohexyl-4,6-dioxohexahydropyrimidine in the yield of 85%. While the hydrogenolysis with the catalyst which was kept standing for 1 or 2 days gave the required product, ECP, in an excellent yield, the use of the freshly prepared catalyst furnished cyclohexenyl compounds as shown by Boon, *et al.*<sup>8)</sup> and when the catalyst which was kept for more than 2 weeks was used, there was obtained the compound which was likely to be a hydrate of ECP.

**On the Metabolic Fate of ECP**—On the metabolic fate of 4,6-dioxohexahydropyrimidine derivatives, it has been described by Butler, *et al.*<sup>9)</sup> that primidon is biotransformed to phenobarbital and *p*-hydroxyphenobarbital. It is easily understood that the methylene group at 2-position of 4,6-dioxohexahydropyrimidine is to be oxidized to a carbonyl group to furnish a barbituric acid derivative. Also, the methylene group neighboring the double bond in a cyclohexene ring is generally oxidized to a carbonyl or hydroxyl group by chromium trioxide.<sup>10)</sup> In cyclohexenyl substituted barbiturates, 3-position of the cyclohexene ring is oxidized to a carbonyl group by chromium trioxide or biotransformed to a carbonyl or hydroxyl group as shown in EHB<sup>1,2)</sup> and MHB.<sup>3)</sup>

The chromium trioxide oxidation of ECP gave a oxo compound which does not possess a dioxo-hexahydropyrimidine ring but a barbituric acid ring. This oxo compound is identical with 3-oxo-EHB which is a chromium trioxide oxidation product of EHB.

8) W. R. Boon, H. C. Carrington, N. Greenhalgh, C. H. Vasey: *J. Chem. Soc.*, 1954, 3263.

9) T. C. Butler: *J. Pharmacol.*, 113, 178 (1955).

10) W. Treibs, H. Schmidt: *Ber.*, 61, 459 (1928).

From the urine of rabbits receiving ECP, 11.5~14.6% of administered drug were recovered and identified by mixed melting point determination, ultraviolet and infrared spectra comparison, and paperchromatography. The isolated metabolite, ECP-M(I), was identified as 3-oxo-EHB on the basis of various physical and chemical data as mentioned above. From these results, it is apparent as shown in Chart 1 that 4,6-dioxohexahydropyrimidine ring is metabolized to the corresponding barbiturate and that a carbonyl group is introduced to 3-position of cyclohexene ring as demonstrated by Tsukamoto, *et al.*<sup>2,3)</sup> and Goldschmidt, *et al.*<sup>11)</sup> in the case of barbiturates. The amounts of both unchanged ECP and ECP-M(I) obtained from the urine were only about 15~20% of medicated ECP. The fate of the remainder was undetectable. It is expected to excrete other metabolites such as 3-hydroxy-EHB, a compound which underwent oxidation in either cyclohexene or dioxohexahydropyrimidine ring, or a ring-destroyed compound. Paperchromatogram of the urine extract showed the presence of another metabolite, which was not yet identified. Thus, it is not yet clear which position of ECP is at first oxidized *in vivo*. Further investigation is now in progress.

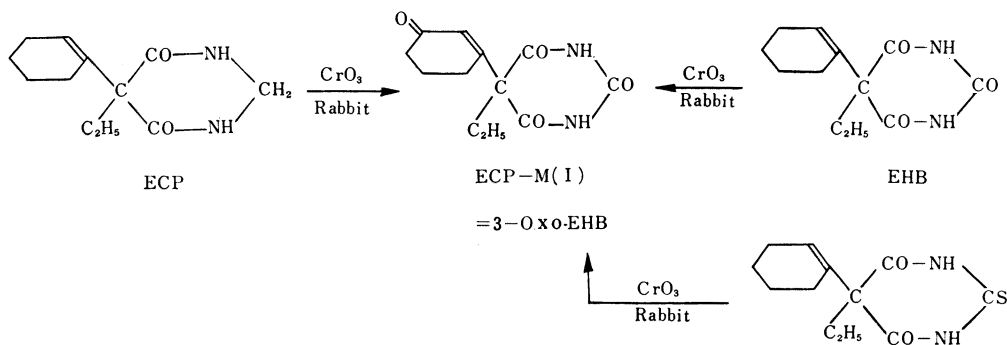


Chart 1. Metabolism of ECP and EHB

**On the Ultraviolet and Infrared Absorption Spectra of ECP and ECP-M(I) or 3-Oxo-EHB**—The ultraviolet absorption peak of ECP appears at 218  $m\mu$  in dehyd. ethanol and at 225  $m\mu$  in 0.5*N* sodium hydroxide solution indicating that ECP forms a monopolar ion. The shift of the peak to a longer wave length in an alkaline solution is due to the double bond. Ferguson<sup>12)</sup> has clarified that the compounds having a  $-C=N-$  unit exhibit an absorption peak at 230~270  $m\mu$  and that the double bond neighbouring to the  $-C=N-$  unit shifts the peak to a longer wave length.

In ethanolic solution, the barbiturates exhibit no absorption maxima but ECP-M(I), or 3-oxo-EHB, in which a carbonyl group has been introduced, exhibits a peak at about 225  $m\mu$  as shown in oxo-MHB.<sup>12)</sup> That this absorption maxima is due to the  $\alpha,\beta$ -unsaturated ketone was discussed in the previous paper.<sup>13)</sup> 2,4-Dinitrophenylhydrazone of 3-oxo-EHB possesses two absorption maxima at 253 and 372  $m\mu$  in ethanolic solution as shown in Table III. This fact agrees with the report of Yaroslavsky<sup>14)</sup> which described that the ultraviolet absorption spectra of 2,4-dinitrophenylhydrazones of  $\alpha,\beta$ -unsaturated carbonyl compounds was characterized by two distinct absorption maxima at 250~260 and 350~400  $m\mu$ . In 0.5*N* sodium hydroxide solution, 3-oxo-EHB exhibits a typical absorption maximum of 5,5-disubstituted barbituric acid, whereas ECP possesses only one absorption maximum at about 225  $m\mu$ .

11) S. Goldschmidt, F.W. Koss: *Z. physiol. chem., Hoppe-Seyler's*, **316**, 224 (1959).

12) L.N. Ferguson, G.E.K. Brauch: *J. Am. Chem. Soc.*, **66**, 1467 (1944).

13) H. Tsukamoto, H. Yoshimura, S. Toki: *This Bulletin*, **4**, 371 (1956).

14) S. Yaroslavsky: *J. Org. Chem.*, **25**, 480 (1960).

Two absorption bands at  $3.51(\nu_{\text{CH}})$  and  $6.01\mu(\nu_{\text{C=O}})$  were observed in the infrared spectrum of ECP and four strong bands at  $5.71$ ,  $5.88$ ,  $5.98$ , and  $6.06\mu$  in that of ECP-M(I) or 3-oxo-EHB. 2,4-Dinitrophenylhydrazone of ECP-M(I) or 3-oxo-EHB absorbs at  $5.88$  and also at  $3.28$ ,  $6.19$ ,  $6.25$ , and  $6.29\mu$  probably due to the vibration of the phenyl group. Both ECP-M(I) and its 2,4-dinitrophenylhydrazone possessed two strong bands between  $11.7$  and  $12.5\mu$  in the finger print region. As mentioned by Levi, *et al.*,<sup>15)</sup> these bands serve to indicate the presence of a barbiturate.

Of the four absorption bands, two bands at  $5.98(\nu_{\text{C=O}})$  and  $6.01\mu(\nu_{\text{C=C}})$  indicated the presence of  $\alpha,\beta$ -unsaturated ketone moiety in the cyclohexene ring because these bands did not occur in ECP and in the 2,4-dinitrophenylhydrazone.

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### Summary

5-Ethyl-5-(1-cyclohexenyl)-4,6-dioxo-hexahydropyrimidine (ECP), which had been expected to display anticonvulsant activity, was prepared by the hydrogenolysis of the corresponding 2-thiobarbituric acid with Raney nickel catalyst, and the mechanism of its biotransformation was discussed.

The transformation of the cyclohexenyl group in 2-thiobarbituric acid derivatives to a cyclohexyl group did not take place when Raney nickel catalyst was prepared by the method of Mozingo.

5-Ethyl-5-(1-cyclohexenyl)-4,6-dioxo-hexahydropyrimidine (m.p.  $269\sim 270^\circ$ ) was obtained in an excellent yields. When administered to rabbits, ECP was partly biotransformed into a metabolite and partly excreted intact in their urine. This metabolite was confirmed to be 5-ethyl-5-(3'-oxo-1'-cyclohexenyl)barbituric acid by admixture with the oxidation product of ethylhexabit with chromimum trioxide. The characterization and identification of the unchanged ECP as well as a metabolite 5-ethyl-5-(3'-oxo-1'-cyclohexenyl)barbituric acid, were also performed by the color reactions with copper-pyridine and cobaltous nitrate-ammonia, by the paper chromatography, and by the measurement of the ultraviolet and infrared absorption spectra.

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