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117. Minoru Yoshimura*2 and Hisao Tsukamoto*3 : Metabolism of Drugs. XXXVII.*1 The Biotransformation of Drugs having Cyclohexene Ring. (4). The Metabolic Fate of 5-Cyclohexenyl-5-alkylhydantoins.

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In the previous papers of this series, the barbiturates[1~3], 2,2-disubstituted glutarimides,[4] and 4,6-dioxohexahydropyrimidine[5] having cyclohexenyl ring have been demonstrated to undergo metabolic oxidation in the position 3 of cyclohexenyl group. It might accordingly be expected that the closely related compounds, 5-cyclohexenyl-5-alkylhydantoins, would undergo the same fate. This report is concerned with the synthesis of 5-cyclohexenyl-5-alkylhydantoins having structural and physiological similarity to the above compounds and the identification of their metabolic products as 5-(3-oxocyclohexenyl)-5-alkylhydantoins.

Experimental*4

2-Cyclohexenyl-2-Ethylcyanoacetamide (IV)——This compound was prepared by the dropwise addition of 42.0 g. of the corresponding chloride in 200 ml. of 28% NH_4OH at room temperature. The product was colorless needles, m.p. 106°. Yield, 30.0 g. *Anal.* Calcd. for $C_{11}H_{16}ON_2$: C, 68.72; H, 8.39; N, 14.57. Found : C, 68.84; H, 8.45; N, 14.22.

2-Cyclohexenyl-2-methylmalonamide——This compound was prepared by the same procedure as applied in the above preparation, using 100 ml. of 28% NH_4OH and 10 g. of the corresponding chloride. Colorless needles, m.p. 146~148°(from EtOH), were obtained from the reaction mixture. This compound was not refined to the extent of analytical purpose. Chloride was prepared by the chlorination of corresponding acid obtained by hydrolysis of nitrilester or of barbituric acid with $SOCl_2$ according to the usual procedure.

Synthesis of 5-Cyclohexenyl-5-Alkylhydantoin (V)——Twenty grams of 2-cyclohexenyl-2-ethylcyanoacetamide or 2-cyclohexenyl-2-methylmalonamide was added to NaOBr solution made from 25.0 g. of NaOH and 11.0 g. of Br_2 in 100 ml. of H_2O. The mixture was vigorously shaken, and heated just below the boiling point for 1 hr. Two milliliters of freshly prepared 10% Na_2S solution was added to the cooled reaction mixture and then filtered. The filtrate was acidified with HCl. The suspending oily substance was taken up by filtration and redissolved in warm 5% NaOH solution. Acidification of the

*1 Part XXXVI : H. Tsukamoto, H. Yoshimura, H. Ide : This Bulletin, **4**, 427(1963).
*2 Showa-Machi, Nagasaki (吉村 実).
*3 Katakasu, Fukuoka (塚元久雄).
*4 All melting points are uncorrected.

1) *a*) H. Tsukamoto, H. Yoshimura : This Bulletin, **2**, 201 (1954); *b*) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **3**, 239 (1955).
2) H. Tsukamoto, H. Yoshimura : *Ibid.*, **3**, 397 (1955).
3) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **4**, 364, 368, 371 (1956).
4) H. Tsukamoto, M. Yoshimura : *Ibid.*, **9**, 581, 584 (1961).
5) *Idem* : *Ibid.*, **10**, 566 (1962).

filtrate with HCl caused the hydantoin to separate as an oily substance which solidified quickly. 5-Cyclohexenyl-5-ethylhydantoin (ECH) (V), recrystallized from hot EtOH, m.p. 197~198°, colorless needles. Yield, 8.0 g. *Anal.* Calcd. for $C_{11}H_{16}O_2N_2$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.64; H, 7.72; N, 13.53.

5-Cyclohexenyl-5-methylhydantoin (MCH) (X) had m.p. 161~163°(from MeOH). Colorless prisms. *Anal.* Calcd. for $C_{10}H_{14}O_2N_2$: C, 61.83; H, 7.27; N, 14.42. Found: C, 62.03; H, 7.24; N, 14.77.

CrO_3 Oxidation of ECH and MCH——The same procedure as mentioned in the previous reports[3~5] was used in this reaction. The product obtained from ECH was colorless plates, m.p. 183°(from EtOH). Yield, 27.0%. *Anal.* Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.54; H, 6.85; N, 12.75.

2,4-Dinitrophenylhydrazone of this compound decomposed at 255~256°. *Anal.* Calcd. for $C_{17}H_{18}O_6N_6$: C, 50.74; H, 4.51; N, 20.89. Found: C, 50.65; H, 4.73; N, 20.25.

On the other hand, the CrO_3 oxidation product of MCH was hard to crystallize, so it was derived to 2,4-dinitrophenylhydrazone with 2,4-dinitrophenylhydrazine sulphate in EtOH. Reddish orange needles, m.p. 235~236°(decomp.). *Anal.* Calcd. for $C_{16}H_{16}O_6N_6$: C, 49.48; H, 4.15; N, 21.64. Found: C, 49.30; H, 4.27; N, 21.26.

The processes of the foregoing reaction are shown in Chart 1.

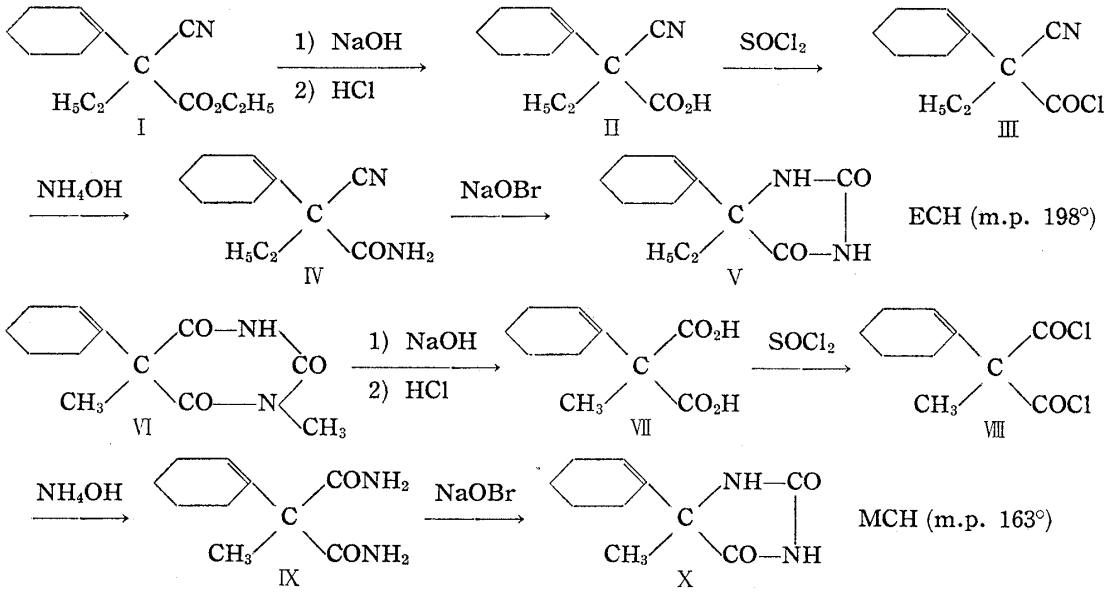

Chart 1. Synthesis of ECH and MCH

The melting points of ECH and MCH were almost in agreement with those reported in the literature.[6]

Synthesis of 5-(3-Oxocyclohexenyl)-5-ethylhydantoin (3-Oxo-ECH)——This was performed by the hydrolysis of 5-(3-Oxocyclohexenyl)-5-ethylbarbituric acid (3-Oxo-EHB), followed by its chlorination, amidation, and ring closure as shown in Chart 2.

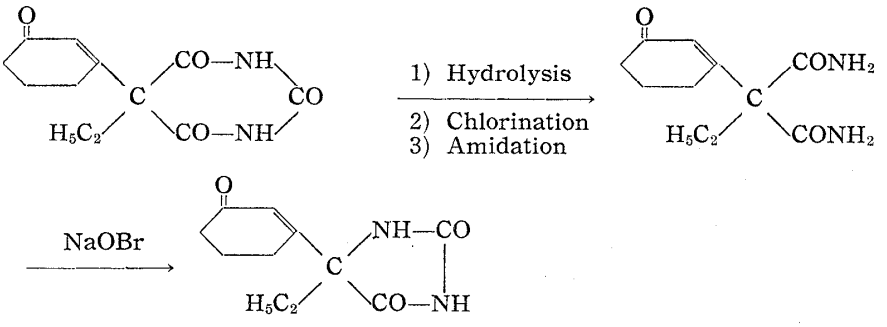

Chart 2. Synthesis of 3-Oxo-ECH from 3-Oxo-EHB

6) Swiss. Patent. 179, 255 (1935), (C.A., **30**, 3591 (1936)).

3-Oxo-ECH was colorless plates, m.p. 181~183°. This melting point was not depressed on admixture with the CrO_3-oxidation product or the urinary metabolite of ECH, m.p. 181~183°.

Isolation of Metabolite from Urine——ECH or MCH at the doses of 150~300 mg./kg. body wt. was orally administered as 1% propylene glycol solution through stomach tube to rabbits weighing 2.5~3.5 kg. and the first 24 hr.'s and the next 24 hr.'s urine were collected separately. At a dose of 300 mg./kg. rabbits slept for 20 hr., but did not sleep with 200 mg./kg. dose. The collected urine was filtered through the cotton, brought to pH 5.0 with conc. HCl and heated on a boiling water bath for 5 hr. to hydrolyze the conjugated metabolites. After cooling, it was continuously extracted with Et_2O for 20~30 hr. The reddish oily residue obtained after the evaporation of the solvent was kept with P_2O_5 in vacuum desiccator over night. The semi-solid residue was treated with $CHCl_3$. The $CHCl_3$ solution was passed through an alumina column, and $CHCl_3$–CH_3OH (9:1) elute was collected. The residue obtained after removal of solvent was dissolved in 10% NaOH solution, and acidified with conc. HCl.

The deposited solid was redissolved in 10% NaOH solution and filtered. The crystallized substance after acidifying of the filtrate with HCl, was repeatedly recrystallized from EtOH to colorless needles, m.p. 183°. The melting point of this metabolite (ECH-M) was undepressed by admixture with 3-oxo-ECH, a CrO_3-oxidation product of ECH. *Anal.* Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.73; H, 6.44; N, 12.84.

The amounts of metabolites of ECH, and MCH isolated are indicated in Table I.

Table I. Isolation of the Metabolite from the Urine of Rabbits Administering ECH or MCH

	Exp. No. of used Rabbits	AV. Body wt. (g.)	Dose (mg./kg.)	Total (mg.)	Vol. of 24 hr.'s Urine (ml.)	Metabolite (mg.)	Metabolite (%)
ECH	2	2,978	300	1,780	260	250.0	14.0
	2	3,100	200	1,240	320	170.0	13.0
MCH	2	3,200	150	960	120	139.0[a]	14.5

[a] This amounts is that of a methanolic Extract.

The 2,4-dinitrophenylhydrazone (DNPH) of ECH-M and MCH-M prepared by the same procedure as that of the CrO_3-oxidation product was decomposed at 255~256° and 235~236°, respectively. The analytical values of these DNPH are shown in Table II.

Table II. The Analytical Data of the DNPH of ECH-M and MCH-M

	m.p. (decomp.) (°C)	Formula	Analytical Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
DNPH of ECH-M	253~256	$C_{17}H_{18}O_6N_6$	50.74	4.51	20.89	51.17	4.76	20.51
DNPH of MCH-M	235~236	$C_{16}H_{16}O_6N_6$	49.48	4.15	21.64	49.13	4.45	21.91

Table III. Paperchromatography of Urine Extracts, Authentic Samples and Isolated Metabolites

Et_2O Extract from Acidified Urine	Rf isoPrOH–$CHCl_3$–conc. NH_4OH (5:4:1)		BuOH saturated with Buffer solution of pH 11	
ECH-administered	0.03[a]	0.35	0.12	0.36
MCH-administered	0.07[a]	0.35	0.17	0.35
Acid Heated Urine				
ECH-administered	0.03[a]	0.35	0.12	0.36
MCH-administered	0.07[a]	0.35	0.16	0.35
ECH-M isolated		0.34		0.36
MCH-M isolated		0.35		0.35
Authentic Samples				
ECH		0.95		0.93
MCH		0.97		0.96
3-Oxo-ECH		0.34		0.36
3-Oxo-MCH		0.35		0.35

a) These spots are likely to be the degradation product of hydantoin ring, respectively.

The melting point (decomp.) of DNPH of those metabolites was undepressed on admixture of corresponding DNPH of the CrO_3-oxidation products of ECH and MCH.

Paperchromatography of the Urine Extracts——Each sample was dissolved in MeOH, their methanolic solution containing 20~45 γ as ECH-M or MCH-M was applied to Toyo Roshi No. 50 filter paper, and run ascendingly for 6 or 15 hr., using the solvent systems of isoPrOH-$CHCl_3$-conc. NH_4OH(5:4:1 v/v) or BuOH saturated borate-NaOH buffer solution of pH 11. The detection of the spots on paper was made by the spray with HIO_4-$KMnO_4$ reagent or with the ethanolic solution of 2,4-dinitrophenylhydrazine sulphate. The Rf values of this ether extracts, summarized in Table III, along with those of the authentic samples and the isolated metabolites.

As shown in Table III, both ether extracts of urine of rabbit administered ECH or MCH indicated the spots having Rf 0.35 or 0.36 in the two developing solvents, respectively. These spots were identical with that of an authentic sample of oxo-ECH or oxo-MCH. On the other hand, the spot having Rf 0.95 or 0.97 corresponding to ECH or MCH itself did not appeared, therefore it was considered that the unchanged ECH or MCH itself had not been contained in these extracts. Moreover, as the spot having Rf 0.03 or 0.07 is likely to indicate the degradation product of hydantoin ring, further investigations are now in progress.

UV Absorption Spectra——The UV absorption spectra were recorded by the Shimazu Photoelectric Spectrophotometer. Comparison of the UV absorption spectra of the extracted ECH-M, the DNPH of ECH-M or of MCH-M, with their synthetic compounds in neutral and alkaline solution are shown in Table IV.

Table IV. Ultraviolet Absorption Spectra of the Extracted and Synthetic Samples

Sample	Medium (10 γ/ml.)	Synthetic sample		Extracted sample	
		λ_{max} (mμ)	log ε	λ_{max} (mμ)	log ε
ECH	Neutral[a]	215	3.56	—	—
	Alkaline[b]	225	4.69	—	—
ECH-M	Neutral[a]	227	3.82	227	3.82
	Alkaline[b]	230	3.90	230	3.90
DNPH of ECH-M	EtOH	255	4.65	255	4.65
		380	6.64	380	6.64
DNPH of MCH-M	EtOH	256	4.65	256	4.65
		379	6.72	379	6.72

a) Ethanolic solution
b) Borate-NaOH buffer (pH 11) solution

The UV absorption spectrum of ECH, as shown in Table IV, showed a maximum at 225 mμ in alkaline medium, and 215 mμ in neutral medium. Contrary to this, the ECH-M or oxo-ECH, in which a carbonyl group had been introduced, exhibited a peak at 230 mμ in alkaline medium and at 227 mμ in neutral medium. These maxima were coincident with the values calculated according to Fieser's rule,[7] therefore, it was assumed that these maxima are due to α,β-unsaturated ketone. Moreover, the 2,4-dinitrophenylhydrazones of ECH-M or MCH-M exhibited two typical absorption peak of 255 and 380 mμ, or 256 and 379 mμ in neutral solution, respectively.

The behavior of the metabolite and the synthetic oxo-compound, and also their 2,4-dinitrophenylhydrazones in the UV absorption spectra was completely identical, respectively.

IR Absorption Spectra——The IR absorption spectra were measured by KBr method in the Koken DC Model 301. Either ECH and MCH showed IR bands at 3.21, 5.68 and 5.88 μ. Whereas the IR spectrum of 3-oxo-ECH revealed absorption bands at 3.21(NH), 5.65, 5.83(>co in hydantoin ring), 5.99 μ ($\nu_{C=O}$) and was superimposable with the spectrum of ECH-M. The IR spectrum of DNPH of 3-oxo-MCH exhibited similar absorption bands at 3.27, 5.68 and 5.81 μ(hydantoin ring), and at 6.19, 6.22 μ (phenyl), 7.44 and 7.61 μ(NO_2) and was identical with the spectrum of DNPH of MCH-M.

Discussion

Urine obtained from rabbits receiving ECH or MCH was heated with acid in the hope of releasing hydroxyl compound from the conjugated form if it might be included in the urine, and a systematic search was made for unchanged material and metabolites by paper chromatography. The result showed that neither unchanged ECH nor

7) L.F. Fieser, M. Fieser: J. Am. Chem. Soc., **71**, 185 (1949).

MCH and nor hydroxylated metabolites could be detected in the urine of rabbit treated with the drugs, as shown in Table Ⅲ.

In addition to it, from the fact that although the metabolite(ECH-M and MCH-M) was isolated from the first 24 hour's urine, no more metabolite was obtained from the next 24 hours' urine, it seems likely to indicate that ECH or MCH was almost metabolized for 24 hours after administration.

The orientation of carbonyl group in ECH-M or MCH-M, as in the closely related compounds, cyclobarbital,[1a,b] hexobarbital,[2,3] cyclohexenylglutarimides[4] and cyclohexenyl 4,6-dioxohexahydropyrimidine,[5] was successfully confirmed to be the position 3 in the cyclohexenyl group. The elucidation of the structures of these metabolites has mainly based on their identity with CrO_3-oxidation products of ECH or MCH, because it is now generally known that CrO_3-oxidation of cyclohexenyl compounds results always in the formation of 3-oxocyclohexenyl derivatives.[1b,3~5] Moreover, chemically known 5-(3-oxocyclohexenyl)-5-ethylbarbituric acid[1b] is unequivocally converted to the 5-(3-oxocyclohexenyl)-5-ethylhydantoin which was shown to be identical with ECH-M as shown in Chart 2.

It is therefore concluded that oxidation on the position 3 in cyclohexenyl group is principal metabolic pathway also in hydantoin compounds having cyclohexene ring.

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Summary

5-Ethyl-, 5-methyl-5-cyclohexenylhydantoins (ECH and MCH), are prepared from the corresponding cyanoacetamide or malonamide using the Hofmann reaction.

These 5,5-disubstituted hydantoins having cyclohexenyl group appear to display narcotic action with 300 mg./kg. in the rabbit. These hydantoins are metabolized to oxo-compounds in the rabbits (about 14.0%), which are identical with the CrO_3-oxidation product of ECH or MCH respectively. It is finally concluded that the biological and chemical oxidation of the drugs having cyclohexenyl group preferentially takes place on the position 3 in the cyclohexenyl group, and the keto formation abolishes the characteristic pharmacological action of the drugs in animal. Accordingly, this biological oxidation reaction is considered to be significant only as a detoxication mechanism.

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