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94. Hisao Tsukamoto*2 and Minoru Yoshimura*3 : Metabolism of Drugs. XXV.*1 Biotransformation of Drugs having Cyclohexene Ring. (2). Metabolic Fate of 2-Substituted Cyclohexenylglutarimides.

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In connection with the metabolic fate of 2-substituted glutarimides, 2-ethylphenylglutarimide (Glutethimid) was investigated by Bernhard[1] and by Hoffmann, *et al.*,[2~4] but 2-substituted cyclohexenylglutarimide has not been studied yet.

In the present series of work, a metabolite of 2-methyl- or 2-phenyl-2-(1-cyclohexenyl)glutarimide was isolated from the urine of rabbits receiving these compounds and they were identified with product of these compounds oxidized with chromic trioxide.

Experimental

2-Methyl-2-(1-cyclohexenyl)glutarimide (MCG) and 2-phenyl-2-(1-cyclohexenyl)glutarimide (PCG) prerared as in the previous work*1 were used.

Extraction of MCG and PCG Metabolites from the Urine of Rabbits——MCG or PCG was administered orally to rabbits in a dose of 250 mg./kg. as the poly(ethylene glycol) solution by stomach tube after emptying the bladder by catheterization. In these doses no hypnotic action was shown. The urine was collected for 48 hr. in a bottle containing a few drops of toluene and filtered through cotton. A total of MCG (1.875 g.) or PCG (2.230 g.) was administered and 900 cc. or 360 cc. of urine was collected, respectively.

Extraction of Free Type Metabolite——A 50-cc. portion of collected urine was extracted with an equal volume of benzene by shaking for 0.5 hr. and extracted with further 25 cc. of benzene. The combined extract was concentrated to about 10 cc. *in vacuo* and dried over Na_2SO_4. The dried extract was chromatographed on alumina and the aliquots of eluates with $CHCl_3$ and $CHCl_3$-MeOH (1:1) were paper chromatographed.

Extraction of Conjugated-type Metabolite——The remaining urine was adjusted to pH 1.0 with conc. HCl and refluxed for 6 hr. to hydrolyze completely the conjugated materials. After cool, the solution was extracted continuously for 30 hr. with Et_2O for MCG and with AcOEt for PCG. A reddish brown oily substance left after evaporation of the solvent was dissolved in Me_2CO and chromatographed on alumina. Unchanged MCG or PCG was recovered from the first $CHCl_3$ eluate and the next elution with $CHCl_3$-MeOH (9:1) gave a small quantity of the metabolite, MCG-M or PCG-M. Aliquots of each eluate were paper chromatographed. On the other hand, a solution of 2,4-dinitrophenylhydrazone sulfate in EtOH was added to the solution of MCG-M or PCG-M and the mixture was warmed on a water bath for 5 min. Reddish orange crystals deposited after cool, were recrystallized from EtOH-AcOEt mixture. 2,4-Dinitrophenylhydrazone of MCG-M and PCG-M decomposed at 230° and 161°, respectively.

Oxidation of MCG and PCG with Chromium Trioxide——To a stirred suspension of 5 g. of powdered MCG or PCG in 40 cc. of Ac_2O, a solution of 5.6 g. of CrO_3 in 40 cc. of Ac_2O was added dropwise at 6~9° during 2 hr. and the reaction mixture was further kept at 15~20° for 1 hr. with stirring, thereafter allowed to stand overnight. The solvent was distilled off to dryness in a reduced pressure and 100~200 cc. of H_2O was added to the residue. This was repeatedly extracted with AcOEt and, after washing with a small quantity of water, the combined extract was dried over Na_2SO_4. The reddish brown oily substance left after evaporation of the solvent, was dissolved in Me_2CO, decolorized through an alumina column, and recrystallized from MeOH. The colorless crystals of oxo-MCG, m.p. 163°, were obtained from MCG and light yellow crystals of m.p. 223°, from PCG.

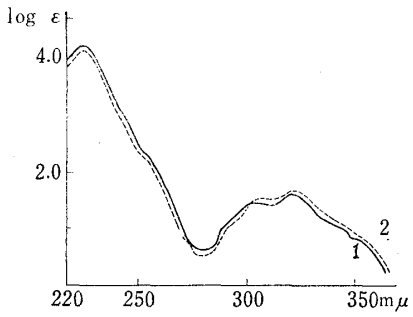
*1 Part XXIV : H. Tsukamoto, M. Yoshimura : This Bulletin, **9**, 581 (1961).
*2 Katakasu, Fukuoka (塚元久雄).
*3 Showa-machi, Nagasaki (吉村 実).
1) K. Bernhard, M. Just, J.P. Vuilleumier, G. Brubacher : Helv. Chim. Acta, **39**, 596 (1956).
2) J. Kebrle, K. Hoffmann : Experientia, **12**, 21 (1956).
3) *Idem* : Helv. Chim. Acta, **39**, 767 (1956).
4) *Idem* : *Ibid.*, **40**, 387 (1957).

The ultraviolet spectra of these oxidation products and metabolites isolated from urine were very similar as shown in Figs. 1 and 2. 2,4-Dinitrophenylhydrazones of these oxidation products showed the same melting points as those of corresponding metabolites isolated from urine and were undepressed on admixture. The analytical data of these hydrazones are shown in Table I and their ultraviolet absorption spectra in EtOH exhibited a typical peak of 2,4-dinitrophenylhydrazone at 390~393 mμ as shown in Figs. 3 and 4.

Table I. 2,4-Dinitrophenylhydrazones of MCG-M, PCG-M, and their Synthesized Compounds

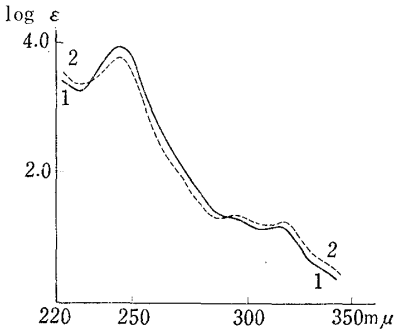
2,4-Dinitrophenylhydrazone of	m.p. (°C)* (decomp.)	λ_{max}^{EtOH} (mμ)	log ε	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
MCG-M (CrO_3-oxidn.)	230	390	4.72	53.86	4.77	17.46	53.73	5.13	17.16
MCG-M (from urine)	230	390	4.97	—	—	—	53.85	5.00	17.54
PCG-M (CrO_3-oxidn.)	161	393	4.82	59.60	4.57	15.11	59.20	4.83	14.64
PCG-M (from urine)	161	391	4.64	—	—	—	58.94	4.86	14.46

* All the melting points are uncorrected.



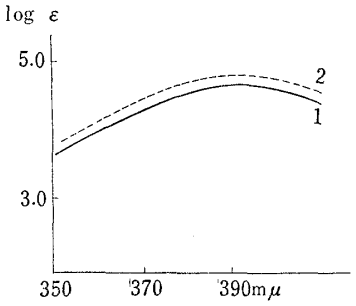
	λ_{max}^{EtOH} (mμ)	(log ε)
1. MCG-M	229	4.149
(Synthesized)	324	1.812
2. MCG-M	229	4.149
(Isolated)	324	1.812

Fig. 1. Ultraviolet Absorption Spectra of MCG-M



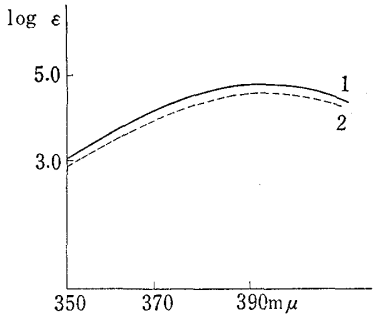
	λ_{max}^{EtOH} (mμ)	(log ε)
1. PCG-M	247	3.920
(Synthesized)	325	1.492
2. PCG-M	247	3.853
(Isolated)	326	1.537

Fig. 2. Ultraviolet Absorption Spectra of PCG-M



1. MCG-M (CrO_3-oxidized)
2. MCG-M (from urine)

Fig. 3. Ultraviolet Absorption Spectra of 2,4-Dinitrophenylhydrazone of MCG-M



1. PCG-M (CrO_3-oxidized)
2. PCG-M (from urine)

Fig. 4. Ultraviolet Absorption Spectra of 2,4-Dinitrophenylhydrazone of PCG-M

Paper Chromatography of Metabolites——Both the aliquots of eluates from alumina column and the synthesized glutarimide derivatives were spotted on a filter paper of Toyo Roshi No. 50 (40×40 cm.) and developed with a 1:1 mixture of $CHCl_3$ and MeOH by the ascending method. The air-dried sheets were sprayed with 0.1% $KMnO_4$ solution and after a few minutes with 1% HIO_4 solution, and then washed with water. The brown color appeared on the zone containing cyclohexene ring. By this method, the glutarimides as little as 75 γ were located. The Rf values of metabolites and synthetic substances are shown in Table II.

Table II. Rf Values of MCG or PCG and their Metabolites

Synthesized sample		Extract for free-type metabolite		Extract for conjugated-type metabolite	
		MCG	PCG	MCG	PCG
MCG	0.98	0.98		0.98	
PCG	0.93		0.98		0.98
Oxo-MCG	0.83	0.83		0.84	
Oxo-PCG	0.78		0.75		0.75
				0.16*	0.07*

* These spots are likely to be of hydroxyl compounds.

From the results of paper chromatography, it is seen that one more metabolite other than unchanged drug and its oxo derivative is present. This is likely to be a hydroxyl derivative but has not been identified as yet.

Properties of Oxo-MCG and Oxo-PCG——These substances are soluble in MeOH, EtOH, iso-PrOH, and Me_2CO, slightly soluble in AcOEt, $CHCl_3$, and Et_2O. The color reaction was positive to alkaline hydroxylamine (2*M* hydroxylamine-HCl, 3.5*N* NaOH), and acidic $FeCl_3$ reagent (3.5*N* HCl, 0.37*M* $FeCl_3$ in 0.1*M* NH_4Cl) or 1% $Co(NO_3)_2$ solution and dil. NH_4OH which are characteristic for glutarimide structure.

Discussion

In the previous works of this series,[5~7] it has been shown that one of the *in vivo* metabolites of barbiturates having a cyclohexene ring was 3-oxo-1-cyclohexenyl compound and it was also prepared chemically by the chromic oxidation of the parent compound.

From the urine of the rabbits receiving MCG or PCG, a metabolite, MCG-M or PCG-M, was isolated and identified with chromic oxidation product (oxo-MCG or -PCG) of MCG or PCG by admixture of their 2,4-dinitrophenylhydrazones and the identical pattern of their ultraviolet absorption spectra.

From the results of color reaction with hydroxamic acid-ferric chloride or cobalt nitrate-ammonia, it is presumed that MCG-M and PCG-M retain the glutarimide structure. The color reaction with the latter reagent especially suggests that hydrogen of imide is ionized, exhibiting acidic reaction. As indicated by Anderson,[8] glutarimide has possibly two forms and it seems likely that they occur together in some kind of an equilibrium.

The decolorization of potassium permanganate solution shows that the double bond in cyclohexene ring remains unchanged in MCG-M and PCG-M. The fact that MCG-M and PCG-M produce 2,4-dinitrophenylhydrazones shows that one carbonyl group has been introduced into MCG and PCG.

As mentioned by Anderson[8] for 2-substituted glutarimide, both MCG and PCG also exhibited no absorption peak even in alkaline solution. The ethanolic solution of 2-substituted glutarimides exhibits no absorption peak because of their non-dissociated forms and even the introduction of a cyclohexene ring contributes little to ultraviolet absorption. However, MCG-M and PCG-M exhibited a peak at 229 and 247 mμ, respectively, and it is presumed that these peaks are due to α,β-unsaturated ketone. In PCG-M, it is influenced by the phenyl group. Furthermore, the evidence that these compounds are α,β-unsaturated ketones was provided by the absorption peak at about 390 mμ of their 2,4-dinitrophenylhydrazone in ethanolic solution. As shown by Bush,[9] *et al.*, the introduction of a carbonyl group into cyclohexene ring was associated with a profound increase in the ultraviolet absorption in both the undissociated and ionic forms. 2-Substituted group, by

5) H. Tsukamoto, E. Takabatake, H. Yoshimura, S. Toki : This Bulletin, **3**, 239 (1955).
6) H. Tsukamoto, *et al.* : *Ibid.*, **4**, 368 (1956).
7) H. Yoshimura : *Ibid.*, **5**, 561 (1957).
8) K.M. Anderson : J. Pharm. Pharmacol., **10**, 242 (1958).
9) M.T. Bush, *et al.* : J. Pharmacol. Exptl. Therap., **108**, 104 ((1953).

virtue of dissociation, depending on their structure, may be the final factor controlling the ultraviolet absorption of the glutarimides at 230 mμ.

It is presumed, as shown in Chart 1, that glutarimides having a cyclohexene ring are oxidized *in vivo* to the corresponding 3-oxo compound in analogy to barbiturates.

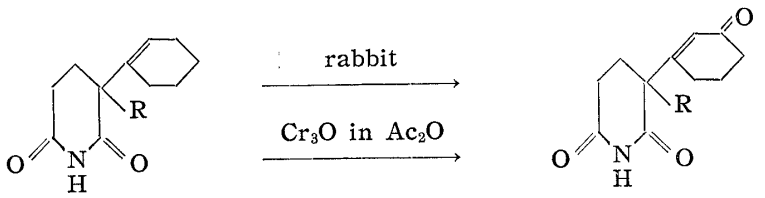


Chart 1.

In paper chromatogram of the extract of conjugated-type metabolite, a spot other than the unchanged compound and oxo-metabolite was found. This metabolite has not yet been isolated but it is likely to be a hydroxyl compound because barbiturates of analogous structure are oxidized to their hydroxyl compound.

The quantitative investigation on the metabolic process of glutarimide having a cyclohexene ring will be made later.

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Summary

The metabolites of 2-methyl-2-(1-cyclohexenyl)glutarimide, and 2-phenyl-2-(1-cyclohexenyl)glutarimide were isolated from the urine of rabbits administered with these compounds and identified with a chromic oxidation product of these compounds. From the ultraviolet absorption spectra, the formation of 2,4-dinitrophenylhydrazone, paper chromatography, and the color reaction, their structures were discussed.

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