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96. Hidetoshi Yoshimura : Metabolism of Drugs. XIII.* The Metabolic Fate of Methylhexabital (5-Cyclohexenyl-3,5-dimethylbarbituric Acid). (5).[1] Further Studies on Isolation, Characterisation, and Identification of Biotransformation Products of the Drug excreted in the Urine of Rabbit.

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Although ethylhexabital (EHB, 5-cyclohexenyl-5-ethylbarbituric acid) and methylhexabital (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid) have analogous chemical structure, earlier studies[2~4] from this laboratory proved that their metabolism in rabbits is very different in respect to their principal metabolites, 3-keto-EHB (5-(3-oxo-1-cyclohexenyl)-5-ethylbarbituric acid) and 3-OH-MHB (5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid). The present report describes further experiments which led to the isolation of additional three metabolites of the drug and establishment of the chemical structures of two of the metabolites.

As pointed out in the previous paper,[4] investigation of MHB-metabolism in dogs by Bush, Butler, and Dickison[5] involved no chemical proof for the structure of the ketone which was principally isolated in this case and the hydroxy compound isolated in this laboratory was not obtained by them. Cooper and Brodie[6] recently indicated that keto-MHB (I) obtained by above workers was also detected by the *in vitro* metabolism of MHB using rabbit liver. Independently from our studies, Tochino[7] isolated only one metabolite of m.p. 208~210° from the urine of rabbits receiving MHB and assumed its structure as hydroxy-MHB by the elementary analysis and infrared absorption spectrum, but similar to the above workers, he did not chemically clarify the structure of the compound.

The discussion on these studies refering to MHB-metabolism is also involved in this paper.

Methods and Results[8]

Isolation of Metabolites of MHB from Rabbit Urine—This procedure was almost analogous to the previous one.[4] A reddish-brown oily substance extracted with AcOEt from the urine of rabbits receiving 10 g. of MHB in several divided doses, same as in previous studies, was dissolved in 50 cc. of AcOEt again, and insoluble matters filtered, which were well washed with AcOEt. The residue left after evaporating the solvent was extracted 3 times with 20 cc. of boiling benzene, and the extract was washed 3 times with a small quantity of water. The washings were evaporated to dryness under reduced pressure and added to the benzene-insoluble portion.

The benzene-soluble substance was dissolved in Me_2CO, chromatographed thorugh an alumina column (1.5×10 cm.), and the eluted substance was recrystallized from MeOH containing a few drops of water to colorless needles, m.p. 112~113°(1-(2-cyclohex-1-enylpropionyl)-3-methylurea[3]).

The substance insoluble in benzene was also dissolved in Me_2CO and chromatographed through an alumina column (1.5×14 cm.), which was eluted with 60~100 cc. of Me_2CO. The effluent was

* This constitutes a part of a series entitled "Metabolism of Drugs" by H. Tsukamoto.
** Katakasu, Fukuoka (吉村英敏).
1) Part (4) : H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, **4**, 371(1956).
2) H. Tsukamoto, E. Takabatake, H. Yoshimura : *Ibid.*, **2**, 201(1954).
3) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **3**, 239(1955).
4) *Idem.* : *Ibid.*, **4**, 368(1956).
5) Bush, Butler, Dickison : J. Pharmacol. Exptl. Therap., **108**, 104(1955).
6) J.R. Cooper, B.B. Brodie : *Ibid.*, **114**, 409(1955).
7) Y. Tochino : Wakayama Igaku (Japan), **6**, 421(1955).
8) All melting points are uncorrected.

fractionated into about 10-cc. portions, and the column was then eluted with MeOH (urea was eluted last). After evaporation of Me_2CO from the eluate, a few drops of MeOH was added to the residue, rubbed with a glass rod, and the crystalline substance formed on standing overnight was separated from the mother liquor.

The mother liquors of each fraction were combined, chromatographed again, and crystalline substances separated in the similar manner as mentioned above. This procedure was repeated several times, until oily substance crystallized almost completely.

In the first and the second chromatographic treatments, majority of crystals consisted only of crude α-3-OH-MHB,[9] as colorless plates, m.p. 170~180°, which were recrystallized from a large quantity of MeOH to colorless columns, m.p. 213~215°(decomp.). In the third, colorless needles, m.p. 145~150°, of crude 3-keto-MHB,[4] were obtained from the earlier eluates, and recrystallized from MeOH containing a few drops of water to colorless plates, m.p. 159~160°. This was followed by MHB-M(IV), as colorless needles, m.p. 190~200°, which were recrystallized from MeOH to colorless plates, m.p. 215~216°(decomp.), from the latter eluates. *Anal.* Calcd. for $C_{11}H_{12}O_4N_2$: C, 55.93; H, 5.08; N, 11.86. Found : C, 56.31; H, 4.96; N, 11.99.

In the fourth chromatography, 3-keto-MHB and α-3-OH-MHB were obtained from the former and the latter eluates, respectively, and in the fifth, 3-keto-MHB was still obtained from the former eluate, but α-3-OH-MHB was no longer obtained. Then, a few drops of AcOEt was added to the fractions of α-3-OH-MHB in the place of MeOH, rubbed with a glass rod, and allowed to stand overnight. The crystallized substance was MHB-M(V), m.p. 110~120°, which was recrystallized from AcOEt to colorless needles, m.p. 143~143.5°; $[\alpha]_D^{16}$ +6.4°(c=2.0, in 95% EtOH). *Anal.* Calcd. for $C_{12}H_{16}O_4N_2$: C, 57.14; H, 6.35; N, 11.11. Found : C, 56.83; H, 6.69; N, 10.80.

In the sixth and the seventh chromatography, MHB-M(VI), m.p. 110~115°, recrystallized from hydr. MeOH to colorless needles, m.p. 122~123°, was obtained from the former eluate, and then 3-keto-MHB, and a mixture of α-3-OH-MHB and MHB-M(V) eluted. By this time, the non-crystallized oily substance remained only in a negligible amount. The yield of these metabolites is shown in Table I.

Table I. Yield of Metabolites of MHB from Rabbit Urine
(Total doses in both experiments were 10.0 g.)

Metabolites \ Expt. No.	1 (mg.)	2 (mg.)
α-3-OH-MHB	500	400
MHB-M(V) (β-3-OH-MHB)	190	250
3-keto-MHB	100	200
MHB-M(IV) (3-keto-nor-MHB)	20	50
Ureide[a]	30	20
MHB-M(VI)	50	30

a) 1-(2-Cyclohex-1-enylpropionyl)-3-methylurea

Characterisation and Identification of the New Metabolites : MHB-M (IV)—MHB-M(IV), colorless plates, m.p. 215~216°(decomp.), was identical with 3-keto-nor-MHB, the oxidation product of nor-MHB[10] by admixture. The elementary analysis was also in agreement with its structure and the ultraviolet absorption spectrum was quite the same as that of 3-keto-nor-MHB.[1] Furthermore the formation of the same 2,4-dinitrophenylhydrazone as those of 3-keto-nor-MHB left no doubt that the structure of MHB-M(IV) was 5-(3-oxo-1-cyclohexenyl)-5-methylbarbituric acid.

MHB-M (V)—MHB-M(V), colorless needles, m.p. 143~143.5°, was oxidized with CrO_3 in cold AcOH solution to colorless plates, m.p. 159~160°, in a good yield, identical with 3-keto-MHB by admixture. It easily formed a *p*-nitrobenzoate, as described below. The ultraviolet absorption spectrum was the same as that of α-3-OH-MHB[1] and the infrared absorption spectrum[11] indicated the existence of a hydroxyl group (2.85 μ) and the characteristic absorption bands[1,12] of barbiturates at 3.11 μ, 3.26 μ (NH), and 5.71, 5.82, and 5.95 μ (c=0) as shown in Fig. 1. Analytical values were also in good agreement with hydroxycyclohexenyl-dimethylbarbituric acid. From these facts, it seemed that the structure would be a diastereoisomer of α-3-OH-MHB, namely β-5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid (β-3-OH-MHB).

9) It corresponds to 3-OH-MHB in the previous paper.[4] Since two diastereoisomeric 3-OH-MHB were isolated as will be described later, α- and β- were prefixed to this compound and the later described one, respectively, for the sake of convenience.
10) H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, 4, 364(1956).
11) This was measured with a Kōken Model DS-201 recording infrared spectrophotometer using NaCl prism.
12) L. Levi, C.E. Hubley : Anal. Chem., 28, 1591(1956).

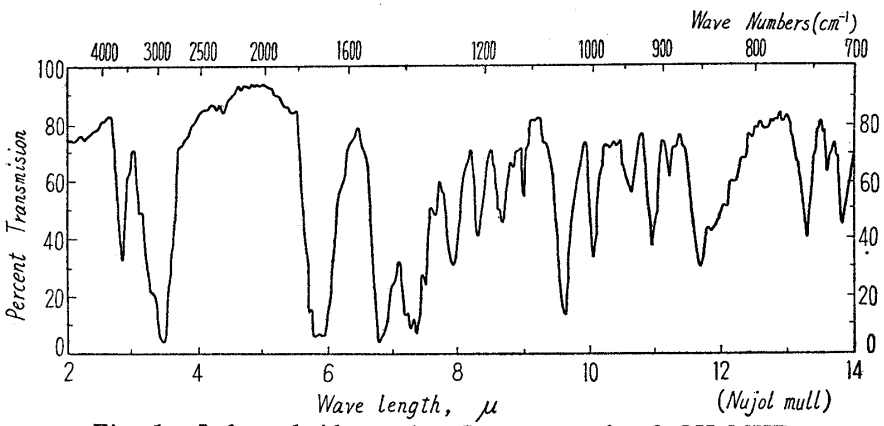

Fig. 1. Infrared Absorption Spectrum of β-3-OH-MHB

Chromic Oxidation of MHB-M (V)—This procedure was analogous to the oxidation of α-3-OH MHB.[4] Yield was about 90%.

***p*-Nitrobenzoate of MHB-M (V)**—This acylation was made in a manner similar to that of α-3-OH-MHB,[4] and recrystallized from MeOH to slightly yellow needles, m.p. 166~167°. *Anal.* Calcd. for $C_{19}H_{19}O_7N_3$: C, 56.86; H, 4.74; N, 10.45. Found: C, 56.48; H, 5.21; N, 10.18. Recrystallization from benzene gave an adduct of one mole of benzene as slightly yellow plates, m.p. 91~93°. *Anal.* Calcd. for $C_{19}H_{19}O_7N_3 \cdot C_6H_6$: C, 62.63; H, 5.22; N, 8.78. Found: C, 63.01; H, 5.35; N, 8.96.

MHB-M (VI)—The structure is not yet established at present.

All the metabolites obtained in this experiment are illustrated in Chart 1.

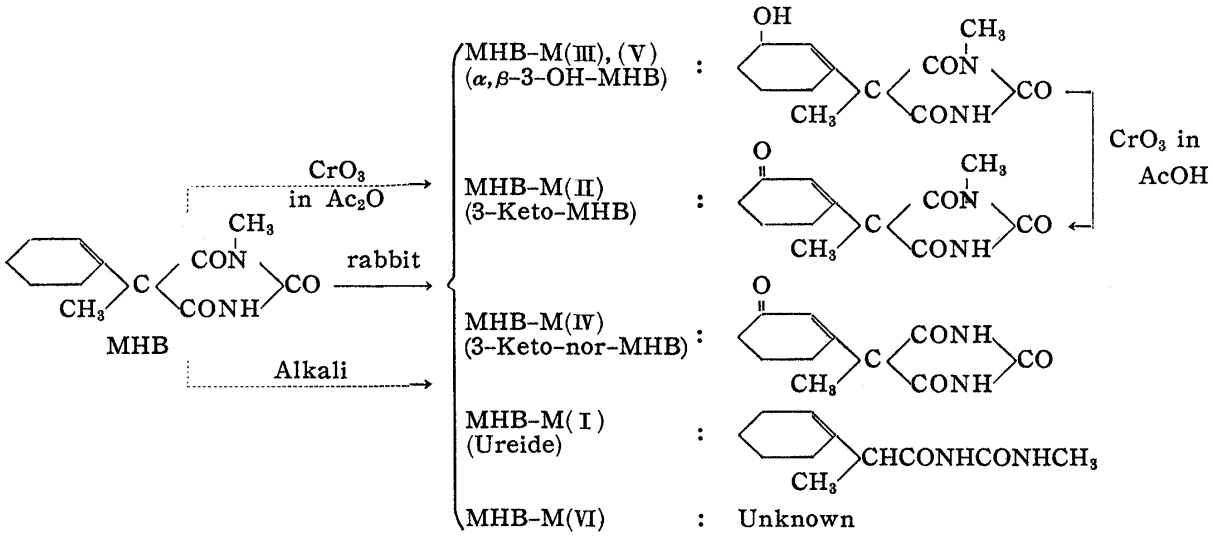

Chart 1.

Discussion

A very interesting result was obtained as to the difference of metabolisms between EHB and MHB in spite of their analogous chemical structures and pharmacological actions. On the one hand, 3-keto-EHB[2,3] was an only metabolite of EHB, and on the other hand, at least six metabolites were excreted in the case of MHB, of which the main metabolites were two diastereisomeric 3-OH-MHB and α-form was predominant over β-form. The total yield of metabolites accounts for only about 10% and the fate of remaining 90% of the administered dose is still unknown.

Bush, *et al.*[5] reported that (A) keto-nor-MHB, m.p. 214~218°(decomp.), (B) keto-MHB (I), m.p. 162~164°, and (C) keto-MHB (II), m.p. 141~142°, were obtained from the urine of dogs receiving MHB, yielding about 5%, 1%, and 0.5% of the doses given, respectively. It was pointed out in the previous paper[4] that (A) and (B) would be

identical with our experimental products, 3-keto-nor-MHB and 3-keto-MHB, respectively, but above workers' presumption on the structure of (C), which was postulated by them to be an isomeric product of (B), would be doubtful from our experimental facts. It seems probable that (C) will be identical with β-3-OH-MHB from the present investigation, in which both melting points of (C) and β-3-OH-MHB are almost identical.

Cooper and Brodie[6] reported that they detected keto-MHB-(I) as a metabolite in the *in vitro* metabolism using rabbit liver by paper chromatographic method and isolated only the same product by counter-current distribution method, but did not obtain 3-OH-MHB. Since 3-keto-MHB and diastereoisomeric 3-OH-MHB have all shown similar Rf values in a paper chromatography[13] in this laboratory, with the solvent system used by them (butanol saturated with 1% ammonium hydroxide), it seems that individual separation from the mixture is considerably difficult and their paper chromatographic results, therefore, imply some doubt with respect to the detection of only 3-keto-MHB.

The fact that 3-keto-nor-MHB has been isolated in this experiment, in spite of no occurrence of nor-MHB, indicates that the rate of oxidation will be much faster than demethylation in the metabolic pathways of MHB, but more detailed experiments are necessary in order to elucidate the metabolic pathways of MHB to oxidation and demethylation products.

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Summary

Additional three metabolites of methylhexabital (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid) were isolated from the urine of rabbits receiving MHB and chemical structures of two of these compounds were established as 5-(3-oxo-1-cyclohexenyl)-5-methylbarbituric acid and β-5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid.

The main metabolites obtained to date were diastereoisomeric 3-OH-MHB [α- and β-5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid, in which α-form was considerably predominant] and 3-keto-MHB [5-(3-oxo-1-cyclohexenyl)-3,5-dimethylbarbituric acid].

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13) These data will not be published now but will be presented in a near future.