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72. Hisao Tsukamoto, Hidetoshi Yoshimura, and Satoshi Toki: Metabolism of Drugs. VII.[1] The Metabolic Fate of Methylhexabital (5-Cyclohexenyl-3,5-dimethylbarbituric Acid). (3).[1] Isolation, Characterization, and Identification of Metabolic Products of Methylhexabital.

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On the metabolic fate of methylhexabital J. P. (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid), the results in outline were reported in the previous communication[2] and the chromic oxidation of MHB and nor-MHB (5-cyclohexenyl-5-methylbarbituric acid) in anhydrous acetic anhydride was previously investigated.[1] Bush, *et al.*[3] reported that two reactions, a demethylation and an oxidation, would probably take place in the body of a dog receiving MHB, and nor-MHB as the demethylating product, and keto-nor-MHB, keto-MHB (I), and (II) as the oxidation products, were isolated, but they did not clarify chemically the structure of the ketones.

Cooper and Brodie[4] recently indicated that keto-MHB (I) was also obtained by the *in vitro* metabolism of MHB using the liver of rabbits. Further, Deininger[5] demonstrated by paper chromatography that demethylation took place in mice injected with MHB.

It is shown in this report that three metabolites of MHB were isolated from the urine of rabbits receiving MHB and their structures were established as cyclohexenylmethyl-N-methylacetylurea, 5-(3′-oxocyclohex-1′-enyl)-, and 5-(3′-hydoxylcyclohex-1′-enyl)-3,5-dimethylbarbituric acid.

Methods and Results**

Isolation of Metabolites of MHB from the Urine of Rabbits—MHB (Oltopan, Dainippon Seiyaku Co., Ltd.) was administered as a freshly prepared aq. solution containing 1.1 equiv. NaOH in doses of about 200 mg./kg. body weight by stomach tube to rabbits after fasting for 24 hrs. and several doses were given over a period of 6 days. It was filtered through the cotton, brought to pH 4.0～5.0 with conc. H_2SO_4, and extracted continuously with AcOEt for 15～18 hrs. The animals were fed *ad lib.* 3 hrs. after the administration and their urine was collected for 24 hrs. A reddish-brown oily substance left after evaporating the solvent was dissolved in acetone, insoluble matters filtered, and the solution was chromatographed through an alumina column.

The first eluate contained a mixture of metabolites and the later one consisted almost of urea. The residue left after evaporation of the solvent from the first eluate was dissolved in a large quantity of water and extracted with benzene.

The matter insoluble in water was also extracted with benzene and the extract was combined with the initial benzene extract. The residue left after evaporation of the solvent from the extract was dissolved in acetone, chromatographed through an alumina columm, and the eluted substance was recrystallized from MeOH containing a few drops of water to colorless needles, MHB-M(I), m.p. 112～113°. *Anal.* Calcd. for $C_{11}H_{18}O_2N_2$: C, 62.86; H, 8.57; N, 13.33. Found: C, 62.94; H, 8.30; N, 12.88.

The aq. solution was evaporated to dryness under a reduced pressure. The oily residue obtained was dissolved in acetone and chromatographed through an alumina column. Colorless needles, MHB-M(II), m.p. 145～150°, and colorless plates, MHB-M(III), m.p. 170～180°, were obtained from a small quantity of the first and the later fraction, respectively. The mother liquor left after the

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1) Part VI: H. Tsukamoto, H. Yoshimura, S. Toki: This Bulletin, **4**, 363(1956).
2) H. Tsukamoto, H. Yoshimura, *Ibid.*, **3**, 397(1955).
3) Bush, Butler, Dickison: J. Pharmacol. Exptl. Therap., **108**, 104(1953).
4) J. R. Cooper, B. B. Brodie: *Ibid.*, **114**, 409(1955).
5) R. Deininger: Arch. exptl. Phathol. Pharmakol., **225**, 127(1955).

** All melting points are uncorrected.

removal of these crystals was again chromatographed and separated into two fractions as above. MHB-M(II) was recrystallized from MeOH containing a few drops of water to colorless plates, m.p. 160~161°. *Anal.* Calcd for $C_{12}H_{14}O_4N_2$: C, 57.60; H, 5.60; N, 11.20. Found: C, 57.59; H, 5.68; N, 11.55.

MHB-M(III) was recrystallized from MeOH to colorless columns, m.p. 213~215°(decomp.); $[\alpha]_D^{14}$ +92.2°(c=0.5, in 95% EtOH). *Anal.* Calcd. for $C_{12}H_{16}O_4N_2$: C, 57.14; H, 6.35; N, 11.11. Found: C, 57.48; H, 6.40; N, 10.71.

The yield of these metabolites is shown in Table I.

TABLE I. Isolation of Metabolites of MHB from the Urine of Rabitts receiving MHB

No. of Rabbits	Av. body wt.	Total dose administered	Yield of Metabolite (mg.)					
			MHB-M(I)		MHB-M(II)		MHB-M(III)	
	(kg.)	(g.)	Crude	Pure	Crude	Pure	Crude	Pure
3~4	2.5	10.480	50	40	100	90	450	300
5~6	2.7	12.600	60	50	160	150	650	500

Characterisation and Identification of Metabolites of MHB: MHB-M (I)—MHB-M (I), colorless needles, m.p. 112~113°, easily soluble in organic solvents, but insoluble in water and even in an alkali. The ultraviolet absorption spectrum had no characteristic absorption band of barbituric acid ring as will be described in the following paper, Part VIII in this series.[6]

From these facts it was considered that the destruction and decarboxylation of the barbituric acid ring would take place and then a derivative of acetylurea would be produced. The elementary analysis was in fair agreement with this presumption.

Previously, Aspelund and Skoglund,[7] and Sato, *et al.*[8] prepared cyclohexenylmethyl-N-methylacetylurea by heating a solution of MHB-Na in a stream of nitrogen. The melting point of 113~114°(reported m.p. 114°(Aspelund) and 117~118°(Sato)) of the substance prepared according to this method was not depressed by admixture with our MHB-M (I).

MHB-M (II)—MHB-M (II), colorless plates, m.p. 160~161°, was identical with the oxidation product (3-keto-MHB (Ia)) of MHB in the preceding paper[1] by admixture and the infrared absorption spectra of these substances were also completely identical as will be described in the following paper.[6] The elementary analysis was also in fair agreement with its structure and further the formation of the same 2,4-dinitrophenylhydrazone as (Ia) left no doubt that the structure of MHB-M (II) was 5-(3′-oxocyclohex-1′-enyl)-3,5-dimethylbarbituric acid.

MHB-M (III)—MHB-M (III), colorless columns, m.p. 213-215°(decomp.), did not form 2,4-dinitrophenylhydrazone but formed *p*-nitrobenzoate, m.p. 202~204°, as described below. The infrared and ultraviolet absorption spectra indicated the characteristic absorption bands of a hydroxyl group and of 5,5,N-trisubstituted barbiturates.

From these facts it seemed reasonable that the structure of MHB-M (III) would be 5-hydroxycyclohexenyl-3,5-dimethylbarbituric acid. The CrO_3 oxidation of MHB-M (III) in glacial AcOH produced in a good yield colorless plates, m.p. 159~160°, identical with MHB-M (II) by admixture, and it was considered that the position of the hydroxyl group in MHB-M (III) was the same as that of the carbonyl in MHB-M (II).

Accordingly the structure of MHB-M (III) was confirmed as 5-(3′-hydroxycyclohex-1′-enyl)-3,5-dimethylbarbituric acid (3-OH-MHB).

***p*-Nitrobenzoate of MHB-M (III)**—To a solution of 100 mg. of MHB-M (III) in 1 cc. of anhyd. pyridine was added 100 mg. of *p*-nitrobenzoyl chloride, allowed to stand overnight at room temp., and 10 cc. of water was added to the solidified reaction mixture. The deposited crystals were collected and recrystallized from MeOH to slightly yellow prisms, m.p. 202~204°. Yield, about 110 mg. *Anal.* Calcd. for $C_{19}H_{19}O_7N_3$: C, 56.86; H, 4.74; N, 10.45. Found: C, 56.80; H, 4.71; N, 10.43.

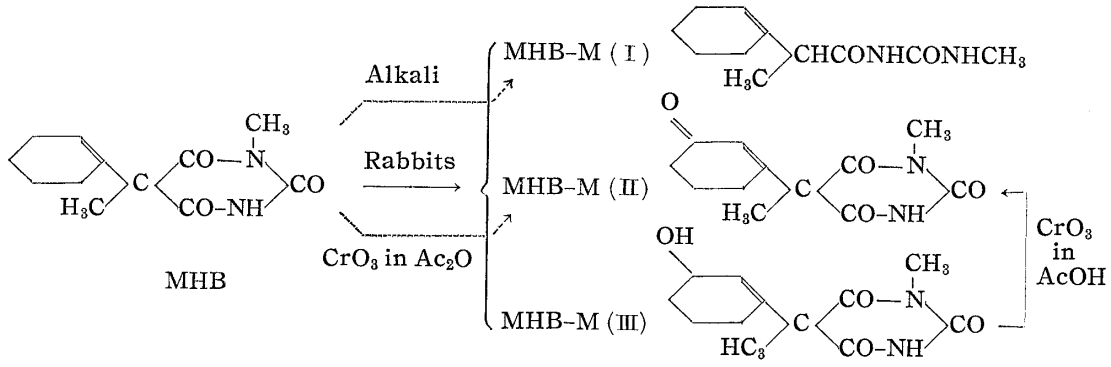
Chromic Oxidation of MHB-M (III)—To a solution of 190 mg. of MHB-M (III) in 2 cc. of glacial AcOH was added a solution of 80 mg. of CrO_3 in 1.5 cc. of glacial AcOH and a drop of water, under shaking for 30 mins. at room temp. (8~9°) and allowed to stand over night.

The green reaction mixture was poured into 3 cc. of water and the solvent was evaporated until dryness under a reduced pressure. To the residue was again added 5 cc. of water, extracted with AcOEt, and the extract was washed with a small quantity of water. After drying over Na_2SO_4, the solvent was evaporated and the residue was recrystallized from MeOH containing a few drops of water to colorless plates, m.p. 159~160°, identical with MHB-M (II) by admixture.

6) Part VIII. H. Tsukamoto, H. Yoshimura, S. Toki: This Bulletin, **4**, 370(1956).
7) H. Aspelund, L. Skoglund: Acta Acad. Aboensis, Math. et Phys., **10**, (10), 22(1937); Chem. Zentr. **108**, II,2004(1937).
8) D. Sato, T. Mineshita, T. Ooka: Ann. Repts. Shionogi Research Lab., **1**, 10(1951).

These three metabolites all have an asymmetric carbon, but only MHB-M (III) was optically active. The foregoing reactions are illustrated in Chart 1.

Chart 1.



Discussion

Hitherto it had been reported by several workers[9~11] that derivatives of malonuric acid, acetylurea, malonamide, and acetamido were produced by the destruction of the barbital ring *in vivo*, but in these cases, either only a negligible amount of the destroyed metabolites were obtained or the destruction had been presumed.

In our experiments on the metabolism of EHB using rabbits,[12,13] these substances were not obtained either. It is notable that the destroyed metabolite, MHB-M (I), is excreted in about 0.5% of the doses adminstered. This fact is interesting in relation to the tendency of easier destruction of the ring of 5,5,N-trisubstituted barbiturate, MHB, than the N-non-substituted derivative, EHB, in alkaline solution. The other significant difference between the metabolism of MHB and EHB is that 3-OH-MHB is principally excreted as well as 3-keto-MHB from the urine of rabbits receiving MHB.

Such an evidence of the barbiturate with cycloalkyl group being oxidized to alcohol is not known so far, although the barbiturates with acyclic alkyl group were shown by several workers to suffer oxidation of the methylene in the acyclic alkyl group to alcohol.[14,15]

Whether, in the oxidation process of MHB *in vivo*, the alcohol is produced intermediately and then oxidized to the ketone or *vice versa* is being examined at present.

Bush, *et al.*[3] previously reported that (A) keto-nor-MHB, m.p. 214~218°(decomp.); (B) keto-MHB (I), m.p. 162~164°; (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. They assumed from the elementary analyses, ultraviolet absorption spectra, and formation of oxime, that these metabolites would be α,β-unsaturated ketones, in which one of the methylene groups of the cyclohexenyl ring is converted to a carbonyl group, although they failed to obtain an oxime from (C).

It seems reasonable that (A) and (B) are identical with our experimental products, 3-keto-nor-MHB and 3-keto-MHB (MHB-M (II)) described in the preceding paper,[1] but (C), which was presumed to be an isomeric product (6-keto-MHB) of (B) by the workers,[3] was not identical with our 6-keto-MHB,[1] m.p. 240~241°, obtained by the

9) van Dyke, Scudi, Tabern : J. Pharmacol. Exptl. Therap., **90**, 364(1947).
10) Maynert, van Dyke : *Ibid.*, **98**, 174, 180(1950).
11) Taylor, Richards, Tabern : *Ibid.*, **104**, 93(1953).
12) H. Tsukamoto, E. Takabatake, H. Yoshimura : This Bulletin, **2**, 201(1954).
13) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **3**, 239(1955).
14) E. W. Maynert, J. M. Dawson : J. Biol. Chem., **195**, 389(1952).
15) E. W. Maynert, J. M. Dawson, E. Washburn : *Ibid.*, **195**, 397, 403(1952).

chromic oxidation of MHB, in respect to the melting point, and therefore their presumption is doubtful.

The main metabolite of MHB, keto-nor-MHB, in dogs was not detected in our experiment using rabbits, and in its place, 3-OH-MHB was obtained as the principal metabolite, yielding about 4~5% of the doses given.

It is interesting that the difference of metabolisms between a dog and rabbit is so remarkable, and in this connection, the same metabolism in a human is now being examined.

Cooper and Brodie[4] reported that keto-MHB (I) was produced by the *in vitro* metabolism using the liver of rabbits, but they did not obtain 3-OH-MHB and the significance of the results by the different experimental conditions between the *in vivo* and *in vitro* metabolism is expected to be settled by future research.

The authors are indebted to Messrs. H. Hattori and K. Funagoshi for the elementary analyses and also to Dainippon Seiyaku Co., Ltd. for their supply of MHB.

Summary

The three metabolites of methylhexabital (5-cyclohexenyl-3,5-dimethylbarbituric acid) were isolated from the urine of rabbits receiving MHB and their structures were established as cyclohexenylmethyl-N-methylacetylurea, and 5-(3′-oxocyclohex-1′-enyl)- and 5-(3′-hydroxycyclohex-1′-enyl)-3,5-dimethylbarbituric acid. Their yield was about 0.5%, 1%, and 5% of the dose administered, respectively.

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73. Hisao Tsukamoto, Hidetoshi Yoshimura, and Satoshi Toki: Metabolism of Drugs. VIII.[2] The Metabolic Fate of Methylhexabital (5-Cyclohexenyl-3,5-dimethylbarbituric Acid). (4). Ultraviolet and Infrared Absorption Spectra of the Metabolites of MHB and Related Compounds.

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As mentioned in the previous papers,[1,2] 5-(3′-oxocyclohex-1′-enyl)-3,5-dimethylbarbituric acid (3-keto-MHB), 5-(6′-oxocyclohex-1′-enyl)-3,5-dimethylbarbituric acid (6-keto-MHB), and 5-(3′-oxocyclohex-1′-enyl)-5-methylbarbituric acid (3-keto-nor-MHB) were obtained by the chromic oxidation of MHB (5-cyclohexenyl-3,5-dimethylbarbituric acid) and nor-MHB (5-cyclohexenyl-5-methylbarbituric acid), and cyclohexenylmethyl-N-methylacetylurea (MHB-M (I)), 3-keto-MHB, and 5-(3′-hydroxycyclohex-1′-enyl)-3,5-dimethylbarbituric acid (3-OH-MHB) were isolated from the urine of rabbits receiving MHB.

The present experiment was undertaken in order to examine the relationship between these compounds and their structures by the ultraviolet and infrared absorption spectra.

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1) Part VI. H, Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, **4**, 363(1956).
2) Part VII. H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **4**, 367(1956).