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3. Hidetoshi Yoshimura: Metabolism of Drugs. XVI.[1] The Metabolic Fate of Methylhexabital (5-Cyclohexenyl-3,5-dimethylbarbituric Acid). (6). Studies on the Reduction of 3-Keto-MHB (5-(3-Oxo-1-cyclohexenyl)-3,5-dimethylbarbituric Acid) and on Pharmacological Activity of the Biotransformation Products of Methylhexabital from the Urine of Rabbits.

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In the earlier papers[2,3] of this series, it was reported that two dextrorotatory hydroxyl compounds were isolated as major metabolites from the urine of rabbits receiving methylhexabital (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid) and their chemical structures were established as diastereoisomeric 5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid (α- and β-3-OH-MHB).

As described in the same papers,[2,3] these hydroxyl metabolites were oxidized to optically inactive 3-keto-MHB (5-(3-oxo-1-cyclohexenyl)-3,5-dimethylbarbituric acid) with chromium trioxide likewise in both cases.

Since two asymmetric carbon atoms are present in 3-OH-MHB, two racemates should be obtained by the reduction of racemic 3-keto-MHB. Thus, in this experiment two hydroxyl compounds, colorless columns (Ia), m.p. 213~215°(decomp.), and colorless needles (Ib), m.p. 143~143.5°, were obtained in good yields by the usual Meerwein-Ponndorf reduction[4] of 3-keto-MHB, in which (Ia) was slightly predominant than (Ib).

(Ia) and (Ib) showed identical melting points respectively with dextrorotatory α-3-OH-MHB, m.p. 213~215°(decomp.), and β-3-OH-MHB, m.p. 143~143.5°, obtained from rabbit urine,[2,3] and also the mixed melting points of each pair showed no depression. Furthermore, both formed *p*-nitrobenzoates, m.p. 202~204° and m.p. 166~167°, respectively, of which each was identical on admixture with the corresponding *p*-nitrobenzoates of α- and β-hydroxyl metabolites.

In addition to above facts, (Ia) and (Ib) were both oxidized to the same 3-keto-MHB and further those were all identical with the corresponding hydroxyl metabolites in the infrared absorption spectra and in other respects, except the optical property (Chart 1).

From these experimental data, it was presumed that two reduction products (Ia) and (Ib) would be the racemic forms of α- and β-3-OH-MHB, respectively, and each pair would form a solid solution.

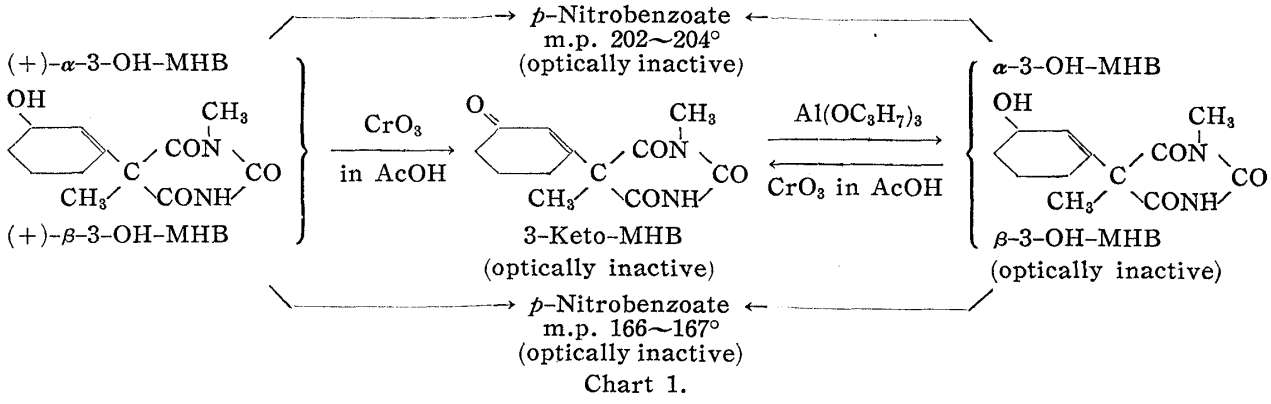

Chart 1.

* Katakasu, Fukuoka (吉村英敏).

1) Part XV. H. Tsukamoto, K. Kato, K. Tatsumi: This Bulletin, **5**, 570(1957).
2) H. Tsukamoto, H. Yoshimura, S. Toki: *Ibid.*, **4**, 368(1956).
3) H. Yoshimura: *Ibid.*, **5**, 561(1957).
4) Org. Reactions, **2**, 178.

In the present experiment the pharmacological activity of four biotransformation products ((+)-α- and (+)-β-3-OH-MHB, 3-keto-MHB, and 3-keto-nor-MHB*) from the urine of rabbits receiving MHB[2,3] was examined by using mice, but all showed no hypnotic action in doses of 400 mg./kg., while the parent compound, MHB, showed a typical hypnotic action in a dose of 100 mg./kg.

In connection with this experiment, Bush, *et al.*[5] previously reported that 3-keto-MHB** and 3-keto-nor-MHB** had both been found inactive as anesthetics in mice in doses of 1 g./kg. intravenously and Tochino[6] indicated that α-3-OH-MHB** had shown no hypnotic action in a dose of 100 mg./kg. in mice as in this experiment, but so far as we know, no studies on β-3-OH-MHB had been made.

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Experimental

Reduction of 3-Keto-MHB—To a solution of 1.0 g. of powdered 3-keto-MHB in 60 cc. of warm *iso*-PrOH, 1.0 g. of Al isopropoxide was added and the mixture was submitted to Meerwein-Ponndorf reduction until the acetone test was completely negative (about 3 hrs.). Thereafter, most of the excess *iso*-PrOH was removed under slightly reduced pressure and the residue was dissolved in about 30 cc. of 5% HCl. The acidic solution was extracted 4 times with 20-cc. portions of AcOEt and the combined extract was dried over anhyd. Na_2SO_4 after washing with a small quantity of water. The residue left after evaporation of the solvent was again dissolved in a small quantity of MeOH and stood for several hrs. About 470 mg. of colorless plates, m.p. 168~173°, crystallized out.

After removal of MeOH from the mother liquor, a few drops of AcOEt was added to it, rubbed with a glass rod, and further ca. 470 mg. of colorless needles, m.p. 138~140°, was obtained.

The former was recrystallized from MeOH to plates, m.p. 180~185°, and further recrystallized several times from a large quantity of MeOH to colorless columns (Ia), m.p. 213~215°(decomp.), and the latter was recrystallized from AcOEt to colorless needles (Ib), m.p. 143~143.5°. *Anal.* Calcd. for $C_{12}H_{16}O_4N_2$: C, 57.14; H, 6.35; N, 11.11, Found (for Ia): C, 57.37; H, 6.35; N, 11.12. Found (for Ib): C, 57.46; H, 6.21; N, 10,68.

***p*-Nitrobenzoates of (Ia) and (Ib)**—These were obtained in a similar manner as described in the previous papers[2,3] and recrystallized from MeOH to slightly yellow prisms, m.p. 202~204°, and slightly yellow needles, m.p. 166~167°, respectively, which were identical on admixture with the corresponding *p*-nitrobenzoates of (+)-α- and (+)-β-3-OH-MHB reported in the previous papers.[2,3]

Chromic Oxidation of (Ia) and (Ib)—By the same tratment as mentioned in the previous papers,[2,3] CrO_3 oxidations of (Ia) and (Ib) both produced in 90% yield, the same colorless plates, m.p. 159~160°, identical with 3-keto-MHB on admixture.

Infrared Absorption Spectra of (Ia) and (Ib) in Nujol—The spectra were obtained with a Kōken Model DS-201 recording infrared spectrophotometer using NaCl prism and were almost identical respectively with those of (+)-α- and (+)-β-3-OH-MHB mentioned in the previous papers.[2,3]

Pharmacological Activity of Four Biotransformation Products of MHB from the Urine of Rabbits—Hypnotic activity of (+)-α- and (+)-β-3-OH-MHB, 3-keto-MHB, and 3-keto-nor-MHB was examined using 5 adult male mice (ddN strain) for each.

All compounds were given intraperitoneally as aqueous solution containing 1.1 equiv. NaOH in a dose of 400 mg./kg., but all caused no hypnotic action. As a control, MHB was also given intraperitoneally in a dose of 100 mg./kg. in 5 mice, and immediately produced a typical hypnotic action lasting about 50 mins. in all mice.

Summary

Two isomeric hydroxyl compounds, m.p. 213~215°(decomp.) and m.p. 143~143.5°, were obtained by reduction of 3-keto-MHB (5-(3-oxo-1-cyclohexenyl)-3,5-dimethylbarbituric acid) and those were identical in all respects with the major urinary metabolites,

* 5-(3-oxo-1-cyclohexenyl)-5-methylbarbituric acid.

** These compounds obtained by above authors were obscure in respect to the chemical structures (see Footnotes 2 and 3).

5) Bush, Butler, Dickinson: J. Pharmacol. Exptl. Therap., **108**, 104(1953).

6) Y. Tochino: Wakayama Med. Repts., **6**, 421(1955).

the diastereoisomeric (+)-α- and (+)-β-3-OH-MHB (5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid), respectively, except the optical property. Pharmacological activity of four main metabolites of MHB obtained previously[2,3] were also examined but none showed any hypnotic action.

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4. Hisao Tsukamoto, Hidetoshi Yoshimura, and Satoshi Toki : Metabolism of Drugs. XVII.[1] Metabolic Fate of Methylhexabital (5-Cyclohexenyl-3,5-dimethylbarbituric Acid). (7). Separation and Identification of Biotransformation Products of Methylhexabital in the Urine of Rabbits by Paper Chromatography.

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It was previously reported[2,3] that ureide (1-(2-cyclohexenylmethylpropionyl)-3-methylurea), two diastereoisomeric 3-OH-MHB (α- and β-5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid), 3-keto-MHB (5-(3-oxo-1-cyclohexenyl)-3,5-dimethylbarbituric acid), 3-keto-nor-MHB (5-(3-oxo-1-cyclohexenyl)-5-methylbarbituric acid), and MHB-M (VI) were obtained from the urine of rabbits receiving methylhexabital (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid).

The present experiment was initiated in order to separate and to identify these compounds by paper chromatography, and then to apply the results to forensic chemistry. These compounds were selectively distributed on buffered filter paper by using butanol buffer as solvent, and this method gave more satisfactory results than butanol-ammonia system[4~10] which had been used in general. Cooper and Brodie[9] investigated the paper chromatography of the metabolic products of Evipal (methylhexabital) by the latter solvent system.

The joint use of sodium metaperiodate and potassium permanganate[11] was taken as spraying reagent for the revelation of spots, and ultraviolet spectrophotometry was used for their characterization.

Materials and Methods**

Materials—MHB (m.p. 142~143°) was supplied by Dainippon Pharmaceutical Co. Ltd., and nor-MHB (m.p. 209~211°(decomp.)) was prepared by the hydrolysis with 5% HCl of 4-imino-5-cyclohexenyl-5-methylbarbituric acid supplied by the same company. 3-Keto-MHB (m.p. 160~161°) and 3-keto-nor-MHB (m.p. 215~216°(decomp.)) were prepared by the oxidation[12] of MHB and nor-MHB, respectively. Ureide (m.p. 112~113°) was prepared by the decomposition[12] of solution of MHB-Na. 3-OH-MHB [α-form, m.p. 213~215°(decomp.), and β-form, m.p. 141~142°(decomp.)] and MHB-M (VI) (m.p. 122~123°) were obtained[2,3] from the urine of rabbits.

Methods—Preparation of Samples—MHB was administered as a freshly prepared aq. solution containing 1.1 equiv. NaOH in a dose of about 200 mg./kg. body weight by stomach tube to a male

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* Katakasu, Fukuoka (塚元久雄, 吉村英敏, 土岐 智). ** All melting points are uncorrected.

1) Part XVI : H. Yoshimura : This Bulletin, **6**, 13(1958).
2) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **4**, 368(1956). 3) H. Yoshimura : *Ibid.*, **5**, 561(1957).
4) E. J. Algeri, J. T. Walker : Am. J. Clin. Pathol., **22**, 37(1952).
5) E. J. Algeri, A. J. McBay : *Ibid.*, **23**, 654(1953).
6) E. J. Algeri, A. J. McBay : Science, **123**, 183(1956).
7) Y. Tochino : Wakayama Med. Repts., **6**, 421(1955).
8) H. Tsukamoto, E. Takabatake, H. Yoshimura : This Bulletin, **2**, 201(1954).
9) J. R. Cooper, B. B. Brodie : J. Pharmacol. Exptl. Therap., **114**, 409(1955).
10) E. Titus, H. Weiss : J. Biol. Chem., **214**, 807(1955).
11) M. L. Wolfrom, J. B. Miller : Anal. Chem., **28**, 1037(1956),
12) H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, **4**, 363(1956).