The authors wish to express their indebtedness to the Research Laboratory of Tanabe Seiyaku Co., Ltd. for the measurement of infrared spectra.

Experimental

Oppenauer Oxidation of Yohimbine—To a mixture of yohimbine (2.0 g.) and $Al(OPh)_3$ (12.0 g.), 50 cc. of purified cyclohexanone (b.p. 156°) and 50 cc. of dehyd. benzene were added, and the mixture was heated at 115~125° in an oil bath for 48 hr. After cool, the resulting reddish brown solution was treated with 10% NaOH solution and the organic layer was shaken with successive portions of 30% H_2SO_4 solution until the aqueous layer no longer produced a precipitate with Mayer reagent. After the combined aq. acid solution was allowed to stand overnight, the resulting white precipitate was collected and washed successively with 10% Na_2CO_3 solution and water.

The same precipitate was also obtained from the mother liquor by addition of 20% Na_2CO_3 solution. This crystalline base was collected and recrystallized from MeOH to faint yellow needles, m.p. 254~255°(decomp.); $[\alpha]_D^{20}$ +15.8°(c=1, pyridine). *Anal.* Calcd. for $C_{21}H_{24}O_3N_2 \cdot CH_3OH$: C, 68.72; H, 7.34; N, 7.29. Found : C, 69.20; H, 6.91; N, 7.67.

Hydrolysis of 17-Ketoyohimbine—A solution of 17-ketoyohimbine (0.03 g.) and KOH (0.01 g.) in EtOH (5 cc.) was refluxed on a water bath for 4 hr. The reaction mixture was evaporated under a reduced pressure, the residue was washed with water, and recrystallized from MeOH. The white crystalline needles melted at 302~303°, undepressed on admixture with an authentic sample of yohimbone.

(Received January 26, 1959)

UDC 591.05 : 615.782.54

Hisao Tsukamoto, Satoshi Toki, and Kyokuritsu Kaneda : Metabolism of Drugs. XX.[1] Metabolic Fate of Methylhexabital (5-Cyclohexenyl-3,5-dimethylbarbituric Acid). (9).[2] Interconversion of 3-Keto-MHB (5-(3-Oxo-1-cyclohexenyl)-3,5-dimethylbarbituric Acid) and 3-OH-MHB (5-(3-Hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric Acid) in the Rabbit.

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In an earlier investigation on the metabolic fate of methylhexabital (MHB, 5-cyclohexenyl-3,5-dimethylbarbituric acid) in the dog, Bush *et al.*[3] have shown that keto-MHB-I and -II, and other metabolites were excreted in the urine. Cooper and Brodie[4] also reported that the same oxidation products were obtained by enzymic biotransformation of MHB. On the other hand, Tochino isolated hydroxy-MHB from the urine of rabbits administered MHB,[5] and confirmed its formation by *in vitro* study using a rabbit liver.[6]

Previous work[7,8] from this laboratory showed that two diastereoisomeric α- and β-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-

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1) Part XIX. H. Tsukamoto, A. Yamamoto : This Bulletin, **7**, 434(1959).
2) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **6**, 88(1958).
3) M. T. Bush, T. C. Butler, H. L. Dickinson : J. Pharmacol. Exptl. Therap., **108**, 104(1953).
4) J. R. Cooper, B. B. Brodie : *Ibid.*, **114**, 409(1955).
5) Y. Tochino : Wakayama Med. Repts., **6**, 421(1955).
6) *Idem.* : *Ibid.*, **7**, 150(1956).
7) H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, **4**, 368(1956).
8) H. Yoshimura : *Ibid.*, **5**, 561(1957).

cyclohexenyl)-5-methylbarbituric acid), ureide (1-(2-cyclohexenylmethylpropionyl)-3-methylurea), and MHB (VI) were excreted from the urine of rabbits administered MHB.

Present investigations were conducted in order to elucidate the metabolic pathway of these products. 3-Keto-MHB, and α- and β-3-OH-MHB were administered individually to rabbits. In all the cases the main urinary products excreted were 3-keto-MHB and 3-OH-MHB. It may be concluded from these observations that these two compounds are interconvertible *in vivo*.

For these purposes, methods of identification and determination of products by buffered paper chromatography, previously reported,[2,9] were adopted.

Materials and Methods*2

3-Keto-MHB (m.p. 160~161°) was prepared by the CrO_3-oxidation of MHB.[10] 3-OH-MHB [α-form, m.p. 213~215°(decomp.); β-form, m.p. 141~142°(decomp.)] were prepared by the Meerwein-Ponndorf reduction of 3-keto-MHB.[11] Extinction measurements were made with a Hitachi photoelectric spectrophotometer with standard 10-mm² quartz absorption cell. Borate-NaOH buffer (pH 10) was prepared by mixing 43.90 cc. solution of 0.1*N* NaOH and 50.00 cc. solution of 0.1*N* H_3BO_3-KCl, then water added to the final volume of 100 cc.

3-Keto-MHB, α-3-OH-MHB, and β-3-OH-MHB were given orally by stomach tube to male rabbits after fasting for 24 hr. as freshly prepared aq. solution containing 1.1 equiv. NaOH in a dose of 200 mg./kg. body weight. These three compounds were given to three rabbits (A, B, and C) alternately, about every 7 days as shown in Table I. The 24-hr. urine was collected and aliquots

Table I. Yield of Metabolites in the 24-hr. Urine of Rabbits administered 200 mg./kg. of 3-Keto-MHB, α-3-OH-MHB, and β-3-OH-MHB

Administered compds.		3-Keto-MHB				α-3-OH-MHB				β-3-OH-MHB			
Rabbit no.		A	C	B	Average	B	A	C	Average	C	B	A	Average
Wt. (g.)		2620	3000	2880									
Exptl. date (1958)		June 17	June 24	July 7		June 17	June 24	July 2		June 17	June 24	July 9	
Urine vol. (cc.)		126	116	160		232	90	194		200	110	110	
Metabolite													
3-Keto-nor-MHB	Concn. (γ/cc.)	—	61.8	—		—	82.7	—		—	93.4	40.9	
	Amt. excreted (mg.)	—	7.2	—		—	7.4	—		—	10.3	4.5	
	Yield (%)	—	1.3	—	0.4	—	1.5	—	0.5	—	1.8	0.9	0.9
3-Keto-MHB	Concn. (γ/cc.)	272.8	377.2	233.0		282.4	535.1	230.9		261.0	342.3	386.7	
	Amt. excreted (mg.)	34.4	43.8	37.3		65.5	48.2	44.8		52.2	37.7	42.5	
	Yield (%)	6.6	7.6	6.2	6.8	11.0	9.3	7.8	9.4	9.1	6.3	8.2	7.9
3-OH-MHB	Concn. (γ/cc.)	488.8	489.4	420.9		578.0	1174.4	780.2		829.8	1667.0	1549.4	
	Amt. excreted (mg.)	61.6	56.8	67.4		134.1	105.7	151.4		166.0	183.4	170.4	
	Yield (%)	11.6	9.9	11.1	10.9	22.4	20.1	26.3	22.9	28.8	30.6	32.5	30.6
Total Yield (%)		18.2	18.8	17.3	18.1	33.4	30.9	34.1	32.8	37.9	38.7	41.6	39.4

of the urine were used for quantitative analysis. The remainders were used for detection and identification of metabolites. These analytical methods were essentially the same as those described in previous papers.[2,9]

Detection and Identification of Metabolites—The bulk of urine was extracted continuously for 15 hr. with AcOEt at pH 4. The residue left after evaporation of the solvent was dissolved in AcOEt. The AcOEt layer was treated with activated charcoal and filtered. The solution was washed with a small quantity of 5% H_2SO_4 and water, and evaporated to dryness. The residue was dissolved in acetone and the solution was chromatographed through an alumina column (alumina,

*2 All melting points are uncorrected.

9) H. Tsukamoto, H. Yoshimura, S. Toki: This Bulletin, **6**, 15(1958).

10) *Idem.*: *Ibid.*, **4**, 364(1956).

11) H. Yoshimura: *Ibid.*, **6**, 13(1958).

1 g., acetone, 50 cc.). The eluted substance was dissolved in about 5 cc. of MeOH and 0.01~0.02 cc. of this solution was applied to paper (Toyo Roshi No. 50) which was previously treated with borate-NaOH buffer (pH 10)*3 and subjected to ascending chromatography for 16 hr. in BuOH saturated with the same buffer as the solvent. For the blank, the 24-hr. urine collected just before the administration of compounds was examined in the same manner as the sample. Authentic samples of 3-keto-nor-MHB, 3-keto-MHB, and α- or β-3-OH-MHB were chromatographed at the same time. The dried paper was sprayed with 1% aq. solution of $NaIO_4$ and 1% $KMnO_4$ solution. Compounds detected were confirmed by ultraviolet spectra.

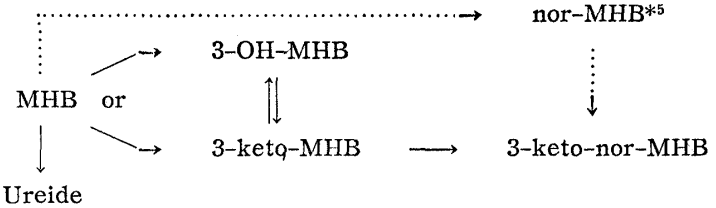
Determination of Metabolites—One cc. of 24-hr. urine was extracted into 12.5 cc. of AcOEt under acid conditions (pH 3) and 10.0 cc. of this solution was evaporated to dryness. The residue was dissolved in 0.5 cc. of MeOH, 0.1 cc. aliquot of this solution was applied to a buffered paper (2×40 cm.) and chromatographed as described above. After development, sections of each metabolite were cut out, eluted with 5.0 cc. of borate-NaOH buffer (pH 11), and the extinction of these solutions was measured at each wave length of maximum optical density (3-keto-nor-MHB : 238 mμ, 3-keto-MHB: 240 mμ, 3-OH-MHB : 244 mμ). Thus the concentration of metabolites was determined. For the blank, the urine collected before the administration of drugs was treated in the same manner.

Results and Discussion

In every case, when rabbits were administered 3-keto-MHB, α-3-OH-MHB, or β-3-OH-MHB, each individually, three transformation products, 3-keto-MHB, 3-OH-MHB,*4 and a negligible amount of 3-keto-nor-MHB, were found in the urine. These preducts exhibit Rf values and characteristic peaks identical with those of the authentic samples. Accordingly, it is supposed that 3-keto-MHB and 3-OH-MHB are exchanged reversibly and that, from the results of quantitative experiments (see Table I), the equilibrium is in favor of 3-OH-MHB. Moreover, it may be clarified that, as shown in the previous paper,[2] the administration of MHB resulted in the predominant formation of 3-OH-MHB.

Analogous reactions were presented in the field of steroid metabolism *in vivo*. For instance, the interconvertibility of cortisone and cortisole[12] is well known and the reaction between 2α-methylcortisone and 2α-methylcortisole[13] has also been demonstrated. Both predonisone and predonisolone[14] have been detected from the urine of humans after administration of either steroid. Thus the 11-ketone and 11β-hydroxy groups in these steroids are interchangeable.

Bush *et al.*[3] suggested that large proportions of keto-nor-MHB must have been produced by the demethylation of keto-MHB and this agrees with observations from the present experimental data.



However, it is uncertain whether the hydroxylation or ketone oxidation occurs first. Accordingly, the metabolic pathway and mechanism of MHB will have to be elucidated by *in vitro* study in the future.

*3 To separate 3-keto-MHB and 3-OH-MHB, buffered paper of pH 10 is preferable to that of pH 11.

*4 The two diastereoisomeric α- and β-3-OH-MHB have same Rf values and cannot be distinguished by the chromatographic procedure used.

*5 Although Bush, *et al.*[3] isolated nor-MHB, it was not obtained in the experiment[7,8] in this laboratory.

12) E. E. Baulieu, M. de Vigan, M. F. Jayle : Comppt. rend. soc. biol., **150**, 882(1956).
13) I. E. Bush, V. B. Mahesh : Biochem. J., **69**, 9p (1958).
14) D. F. Johnson, E. Heftman, J. J. Bunim : Proc. Soc. Exptl. Biol. Med., **94**, 291(1957).

Summary

In order to elucidate the transformation processes of MHB metabolites, 3-keto-MHB, α-3-OH-MHB, and β-3-OH-MHB were administered to rabbits. 3-Keto-nor-MHB, 3-keto-MHB, and 3-OH-MHB have been identified in every case from the 24-hour urine by buffered-paper chromatography. From these and the results of quantitative studies, it is concluded that the 3-ketone and 3-hydroxyl groups in the cyclohexenyl ring are interconvertible and the equilibrium is in favor of 3-OH-MHB.

(Received January 28. 1959)