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132. **Hisao Tsukamoto and Yukio Kuroiwa :** Metabolism of Drugs. XXI.[1] Isolation and Detection of Hydroxyl Derivatives from the Urine of Rabbits receiving Ethylhexabital (EHB, 5-Cyclohexenyl-5-ethylbarbituric Acid) and Normethylhexabital (Nor-MHB, 5-Cyclohexenyl-5-methylbarbituric Acid).

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It has been reported that 3-keto-EHB (5-(3-oxo-1-cyclohexenyl)-5-ethylbarbituric acid) was isolated from the urine of rabbits receiving EHB (ethylhexabital, 5-cyclohexenyl-5-ethylbarbituric acid)[2,3] but MHB (methylhexabital, 5-cyclohexenyl-3,5-dimethylbarbituric acid) was biotransformed into various compounds such as 3-OH-MHB, 3-keto-MHB, 3-keto-nor-MHB, etc.[4,5] In the metabolism of EHB, 3-OH-EHB (5-(3-hydroxy-1-cyclohexenyl)-5-ethylbarbituric acid) was detected from the urine of man receiving EHB, by using buffered paper chromatography as described previously[6] and isolated from the urine of rabbits as shown in this paper. It was also found that hydroxyl derivative was excreted in the case of nor-MHB.

Materials and Methods

EHB (m.p. 170∼174°) was liberated from EHB-Ca[*2] with 10% HCl, extracted with ether, and recrystallized from boiling water. Nor-MHB (m.p. 209∼211°) was prepared by hydrolysis of 4-imino-5-cyclohexenyl-5-methylbarbituric acid[*3] with 5% HCl. 3-Keto-nor-MHB (m.p. 214∼215°) was obtained from the urine of rabbits receiving nor-MHB and identical with the synthesized product[7] because of no depression of melting point on admixture. 3-OH-nor-MHB (m.p. 194°) was prepared by the Meerwein-Ponndorf reduction of 3-keto-nor-MHB.

Isolation of 3-OH-EHB from Urine—EHB (14 g. as total) was administered as an aqueous solution containing 1.1 equiv. of NaOH in a dose of about 200∼300 mg./kg. of body weight by stomach tube to male rabbits after fasting for 12 hr. over the period of 7 days. The 24-hr. urine was collected, filtered through cotton, brought to pH 2∼3 with conc. H_2SO_4, and extracted continuously with AcOEt for 16∼18 hr. The AcOEt extract was separated and washed with a small quantity of 5% H_2SO_4 and water, and dried over anhyd. Na_2SO_4. After evaporation of AcOEt, 3-keto-EHB was recrystallized from MeOH and removed as much as possible. The residue obtained by evaporation of MeOH was dissolved in acetone and chromatographed through an alumina column. The substance which was eluted first was crystallized from AcOEt. To 200 mg. of crystals (m.p. 190∼200°) dissolved in 3 cc. of MeOH a solution of 150 mg. of Girard P reagent[8] dissolved in 0.3 cc. of glacial AcOH was added and refluxed for 1.5 hr. in a boiling water bath. After cool, the reaction mixture was poured into ice water and extracted with 100 cc. of ether. After the removal of ether dried over anhyd. Na_2SO_4, the residue was recrystallized from AcOEt. The crystals were dissolved in acetone, again chromatographed through an alumina column, and recrystallized from AcOEt to colorless plates, m.p. 185∼186°; yield, 50 mg.

A solution of 50 mg. of crude 3-OH-EHB, obtained from the urine of rabbits receiving a total

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[*2] This material was supplied from Shionogi & Co. Ltd.

[*3] This material was supplied from Dainippon Pharmaceutical Co., Ltd.

1) Part XX. H. Tsukamoto, S. Toki, K. Kaneda : This Bulletin, **7**, 651(1959).

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) H. Yoshimura : *Ibid.*, **5**, 561(1957).

6) H. Tsukamoto, S. Toki, K. Kaneda : *Ibid.*, **5**, 625(1957).

7) H. Tsukamoto, H. Yoshimura, S. Toki : *Ibid.*, **4**, 364(1956).

8) A. Girard, G. Sandulesco : Helv. Chim. Acta, **19**, 1095(1936).

of 5 g. of EHB dissolved in 1 cc. of anhyd. pyridine and added with 50 mg. of *p*-nitrobenzoyl chloride, was allowed to stand over night at room temperature, and 5 cc. of water was added to the solidified reaction mixture. The deposited crystals of *p*-nitrobenzoate of 3-OH-EHB were collected and recrystallized from MeOH to slightly yellow prisms of m.p. 201°; yield, 35 mg. *Anal.* Calcd. for $C_{19}H_{19}O_3N_7$: C, 56.85; H, 4.77; N, 10.47. Found : C, 56.90; H, 4.74; N, 10.47.

Synthesis of 3-OH-EHB—To a solution of 1 g. of powdered 3-keto-EHB in 60 cc. of warm *iso*-PrOH, 1 g. of $Al(iso\text{-}PrO)_3$ was added and the mixture was heated in an oil bath at 105~115° until the acetone test became completely negative (about 3 hr.). Thereafter, most of the excess *iso*-PrOH was removed under slightly diminished pressure and the residue was dissolved in 20 cc. of 5% HCl. The acid solution was extracted 3 times with 15-cc. portions of AcOEt and the combined extract was dried over anhyd. Na_2SO_4. The residue left after evaporation of the solvent was again dissolved in a small quantity of AcOEt and recrystallized from AcOEt to white plates, m.p. 184~185°; yield, 0.4 g. *Anal.* Calcd. for $C_{12}H_{16}O_4N_2$: C, 57.14; H, 6.35; N, 11.11. Found : C, 57.15; H, 6.11; N, 11.32.

p-Nitrobenzoate of the reduction product was prepared same as the metabolite, as slightly yellow prisms of m.p. 201°. Yield, about 50%. *Anal.* Calcd. for $C_{19}H_{19}O_7N_3$: C, 56.85; H, 4.77; N, 10.47. Found : C, 56.90; H, 5.04; N, 10.16.

Both biological metabolite and synthetic product were apparently identical because of no depression in the melting point on admixture of their *p*-nitrobenzoate and of identical pattern in the ultraviolet spectra.

Quantitative Determination of the Metabolites in the Urine—EHB or nor-MHB was given as described above and the 24-hr. urine was collected and filtered through Hyflo Super Cel. In a glass-stoppered tube were placed 1 cc. of urine, 0.5 cc. of $0.2N$ H_2SO_4 (adjusting pH of medium to 5.0), and 12.5 cc. of AcOEt. After shaking the mixture, 10 cc. of AcOEt phase was pipetted out and evaporated to dryness. The residue was dissolved in 0.8 cc. of MeOH and 0.02 cc. of this solution was applied on a paper treated with the borate-NaOH buffer of pH 10 by the same procedure as previously reported.[9~11] The developing solvent was BuOH saturated with borate-NaOH buffer of pH 11 and ascending method was used. From the paper strip of the urine sample, two portions were cut out corresponding to the spots located by spraying of 1% $NaIO_4$ and 1% $KMnO_4$ on the paper. This was chromatographed with authentic samples (Fig. 2). These area were Rf. 0.1~0.2 for 3-Keto-EHB and 0.2~0.4 for 3-OH-EHB. The paper section was eluted with 5 cc. of borate-NaOH buffer of pH 11 and its absorption was measured at 230, 239, and 250 mμ by a spectrophotometer. The maximum absorption of ultraviolet for EHB, 3-keto-EHB, and 3-OH-EHB at pH 11 is at 239 mμ and nor-MHB, 3-keto-nor-MHB, and 3-OH-nor-MHB have absorption peak at 238 mμ (Fig. 1). From the optical density at 239 mμ the amounts of EHB metabolites were determined. Separation of the metabolite of nor-MHB was performed in the same way and spectrophotometry was carried out at 238 mμ.

Results and Discussion

3-OH-EHB was isolated from the urine of rabbits receiving EHB other than 3-keto-EHB. The reduction of 3-keto-EHB with aluminium isopropoxide gives a product which is identical with the metabolite mentioned above, there being no depression of melting point on admixture and same pattern of ultraviolet and infrared spectra. The spots corresponding to 3-OH- and 3-keto-EHB were found in the urine of rabbit, using the buffered paper chromatography, but the unchanged drug was not found.

On the metabolism of nor-MHB, Bush, *et al.*[12] reported that unchanged nor-MHB and keto-nor-MHB were obtained from the urine of dogs receiving nor-MHB, yield being about 13% and 20% of the doses given, respectively. In the present investigation it was found that a new metabolite, 3-OH-nor-MHB, was excreted in the rabbit urine besides above two compounds. The chromatogram of a new spot corresponding to authentic sample of 3-OH-nor-MHB was cut out after paper chromatography of the urine extract of rabbit receiving nor-MHB and eluted with borate-NaOH buffer of pH 11. The ultraviolet spectrum of this eluate was completely identical with that of synthetic sample. The results of estimation of these metabolites excreted in the urine are shown in Table I.

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9) H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, **6**, 15(1958).
10) *Idem.* : *Ibid.*, **6**, 88(1958).
11) H. Tsukamoto, E. Takabatake, T. Ariyoshi : *Ibid.*, **3**, 459(1955).
12) M. T. Bush, T. C. Butler, H. L. Dickson : J. Pharmacol. Exptl. Therap., **108**, 104(1953).

Table I. Yield of Various Metabolite in the 24-hr. Urine of Rabbits

Dose of EHB (mg./kg.)		200			300		
Rabbit No.		A	B		A	B	
Body weight (kg.)		2.55	2.8		2.5	2.88	
Urine vol. (cc.)		123	271		129	117	
Metabolite							
3-keto-EHB	Amt. excreted (mg.)	88	180		189	287	
	Yield (%)	17.2	32.1	24.6	25.2	33.5	29.8
3-OH-EHB	Amt. excreted (mg.)	83	165		158	236	
	Yield (%)	16	29.4	22.7	21	27.6	24.1
Total yield (%)		33.2	61.5	47.3	46.2	61.1	53.9
Dose of nor-MHB (mg./kg.)		200			300		
Rabbit No.		A	B		A	B	
Body weight (kg.)		3.63	3.00		3.06	3.00	
Urine vol. (cc.)		260	147		262	202	
Metabolite							
3-keto-nor-MHB	Amt. excreted (mg.)	220	176		241	221	
	Yield (%)	30.5	29.3	29.9	26.8	25	25.9
3-OH-nor-MHB	Amt. excreted (mg.)	142	105		149	135	
	Yield (%)	19.7	17.5	18.6	16.6	15	15.8
nor-MHB (unchanged)	Amt. excreted (mg.)	8.8	17.7		32.4	40.2	
	Yield (%)	1.2	2.9	2.1	3.6	4.4	4.0
Total yield (%)		51.4	49.7	50.6	47	44.4	45.7

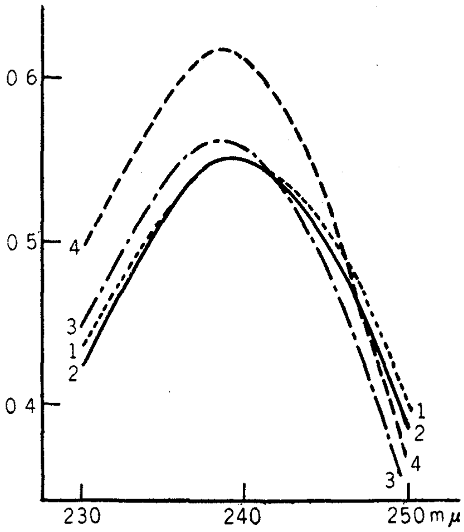

Fig. 1. Ultraviolet Spectra of Hydroxyl Derivatives in Borate-NaOH Buffer Solution (pH 11)

1) 3-OH-EHB (isolated)
2) 3-OH-EHB (synthetic)
3) 3-OH-nor-MHB (synthetic)
4) 3-OH-nor-MHB (eluted from paper)

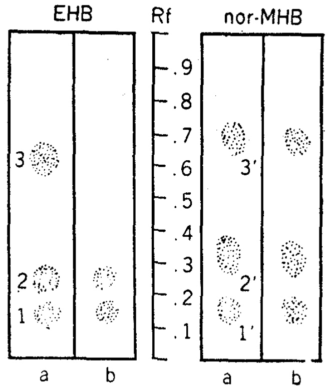

Fig. 2. Buffered-paper Chromatogram of Metabolites in Urine

a : Authentic samples of (1) 3-keto-EHB, (2) 3-OH-EHB, (3) EHB, (1′) 3-keto-nor-MHB, (2′) 3-OH-nor-MHB, (3′) nor-MHB
b : Test samples

It was found that 3-OH-EHB and 3-OH-nor-MHB were inactive as hypnotic by the intraperitoneal injection in doses of over 1 g./kg. of body weight in mice. The results obtained show difference of metabolism between EHB or nor-MHB and MHB in spite of their analogous chemical structures and pharmacological actions. On the one hand, 3-keto-EHB and 3-keto-nor-MHB were the main metabolite of EHB and nor-MHB, respectively, and on the other hand, at least six metabolites were produced in the case of MHB in which the main metabolites were two diastereoisomeric 3-OH-MHB.[4,5]

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Summary

3-OH-EHB was isolated from the urine of rabbits receiving EHB besides 3-keto-EHB and identified with the reduction product of 3-keto-EHB. After the administration of nor-MHB to rabbits, 3-OH-nor-MHB was also detected from the urine, using buffered paper chromatography. Both 3-OH-EHB and 3-OH-nor-MHB have no hypnotic action.

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133. **Tsutomu Momose, Yo Ueda, and Tatsuo Shoji :** Organic Analysis. XIV.[*1] Infrared Spectra of Phenylsulfonyl Derivatives. (3). The C-H Deformation Vibrations of Benzene Ring, the CH_3 Rocking Frequencies of SO_2CH_3 Group, and the Characteristic Absorption Bands of SO_2NH_2 Group.

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In the previous papers[1,2] of this series, the substituent effect on the SO_2-stretching frequencies and the CO-stretching frequency of the N-acetylsulfonamide group were described. In this work, the other absorption bands of phenylsulfonyl derivatives in the regions of 9～13 and 6～7 μ are discussed, dealing with 50 kinds of phenylsulfonyl, 52 kinds of benzenesulfonamide, and 6 kinds of aliphatic sulfonamide derivatives.

Experimental

Most of the spectra measured and reported in the previous papers were utilized in this work. Several new spectra were measured as liquid or Nujol mull by Koken DS-201 or DS-301 Infrared Spectrophotometer using NaCl prism.

Results and Discussion

C-H In-plane Deformation Vibration

All phenylsulfonyl compounds measured generally exhibited strong absorption bands in the region of 1106～1078 cm^{-1} (9.04～9.28 μ). Their frequencies are tabulated in the first column of Tables I and II, and typical curves are shown in Fig. 1. Two *ortho*-substituted derivatives showed the same bands at 1129 and 1120 cm^{-1}.

Baxter, *et al.*[3] already found these bands in several phenylsulfonyl derivatives and assigned them to S-N stretching vibration. In N-methyltoluene-*p*-sulfonamide, Hadži[4]

[*1] Part XVIII : This Bulletin, **7**, 31(1959).
[*2] Katakasu, Fukuoka (百瀬勉, 上田 陽, 庄司達雄).
1) T. Momose, Y. Ueda, T. Shoji : This Bulletin, **6**, 415(1958).
2) T. Momose, Y. Ueda, T. Shoji, H. Yano : *Ibid.*, **6**, 669(1958).
3) J.N. Baxter, J. Cymerman-Craig, J.B. Willis : J. Chem. Soc., **1955**, 669.
4) D.H. Hadži : *Ibid.*, **1957**, 847; S. Detoni, D.H. Hadži : Spectrochim. Acta, **9**, 601(1957).