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.

(Received October 4, 1957)

UDC 615.782.54-092.21

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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rabbit after fasting for 24 hrs. The 24-hr. urine was collected, filtered through a cotton, brought to pH 4~5 with conc. H_2SO_4, and extracted continuously with AcOEt for 15 hrs. The residue left after evaporation of the solvent was dissolved in AcOEt, insoluble matters filtered, and the solution was washed with a small quantity of 5% H_2SO_4 and water. The solvent was evaporated to dryness, the residue was dissolved in acetone, insoluble matters filtered, and the solution was chromatographed through an alumina column (alumina 5 g., acetone 50 cc.) in order to remove a persistent impurity. The eluted substance was dissolved in about 10 cc. of MeOH, insoluble substances were filtered, the solution was concentrated to about 5 cc., and a portion was applied to paper for chromatography. For the blank, the urine collected before administration of the drug was treated in the same manner as above.

Chromatographic Technique—The development was made by the ascending method in a chamber, 44 cm. in height and 6 cm. in diameter. Rectangular strip of Toyo Roshi No. 50 (12×40 cm.) was sprayed with the borate-NaOH buffer (pH 11). The paper was left suspended in air by the edge and used as soon as it dried. The MeOH solution of the testing sample, the authentic samples and the blank described above, was applied simultaneously at intervals of 3 cm. along the base line 7 cm. from the bottom edge of the sheet. Quantities of 50~100 γ, in a volume of 0.005 to 0.02 cc. (usually 0.01 cc.), were used for detection of metabolites of MHB and establishment of Rf values.

The paper was hung in a chamber containing BuOH saturated with borate-NaOH buffer (pH 11) (1 : 1, upper layer) and allowed to remain in equilibrium with the atomosphere therein for at least 1 hr. before the paper was immersed in the solvent. The development was accomplished within a period varying from 16 to 18 hrs., with the overnight period often being convenient. The filter paper was then dried in air as rapidly and uniformly as possible in order to prevent spreading of the spots. It was first sprayed with 1% aq. solution of $NaIO_4$, and after 3~4 mins., freshly prepared 1% aq. solution of $KMnO_4$ was sprayed. The paper was then washed with water until the excess of permanganate was removed. This treatment will frequently reveal faint brown spots on a white background. The area of each spot was cut out, eluted with borate-NaOH buffer (pH 11), and the solution was submitted to ultraviolet spectrophotometry. Their wave length of maximum optical density was identical with that of authentic samples.[13]

Results and Discussion

Five spots corresponding to ureide (λ_{max}–), α– and β–3–OH–MHB (λ_{max} 244 mμ*), 3–keto–MHB (λ_{max} 240 mμ), 3–keto–nor–MHB (λ_{max} 238 mμ), and unknown compounds**

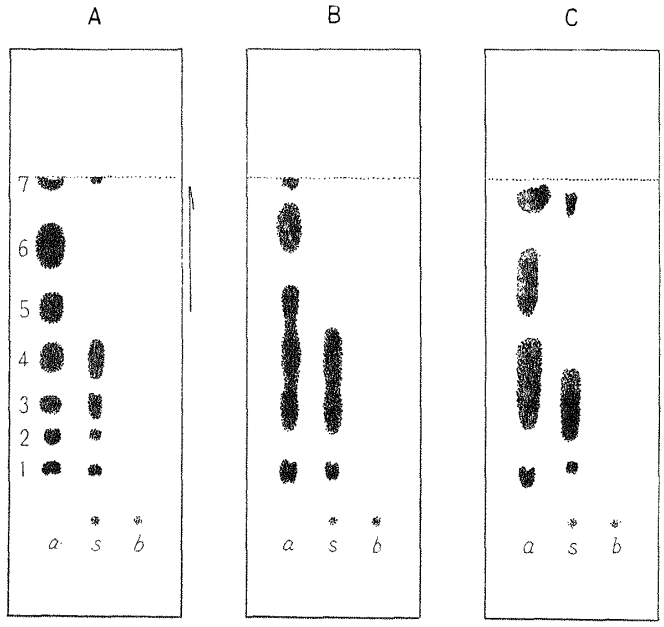

Fig. 1.

A. Buffered filter paper.
Solvent, BuOH–borate buffer.

B. Unbuffered filter paper.
Solvent, BuOH–0.5*N* NH_4OH.

C. Unbuffered filter paper.
Solvent, BuOH–5*N* NH_4OH.

a. Authentic samples. 1, 3-Keto-nor-MHB; 2, MHB-M(VI); 3, 3-Keto-MHB; 4, 3-OH-MHB; 5, Nor-MHB; 6, MHB; 7, Ureide.

s. Test samples.

b. Blank.

* The ultraviolet spectra of the two substances were identical and exhibited characteristic peak at 244 mμ.

** The ultraviolet absorption spectrum of the eluate of this spot exhibited a peak at about 244 mμ although its extinction was very low, which had similar characteristic peak with MHB-M (VI) (m.p. 122~123°, λ_{max} 244 mμ) obtained from the urine of rabbits administered with MHB, and their Rf value agreed closely.

13) H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, 4, 371(1956).

Table I. Rf Values of MHB Metabolites and Related Compounds

Compound \ Solvent system	BuOH–borate[b] buffer	BuOH–0.5N NH_4OH	BuOH–5N NH_4OH
Ureide	0.93	0.97	0.91
3-OH-MHB[a]	0.43	0.46	0.34
3-Keto-MHB	0.29	0.37	0.30
MHB-M (VI)	0.26	—	—
3-Keto-nor-MHB	0.13	0.15	0.14
MHB	0.76	0.73	0.73
Nor-MHB	0.58	0.53	0.53
6-Keto-MHB	0.49	0.48	0.30

Toyo Roshi No. 50 filter paper (1×40 cm.). Authentic samples were applied, 50~100 γ.
a) 3-OH-MHB included α- and β-forms, both of which had the same Rf value.
b) Using the buffered filter paper.

were found in the urine of rabbits receiving MHB (Fig. 1). Table I shows Rf values of MHB metabolites and related compounds using the three solvent systems.

At first, saturated aqueous solution of mercuric chloride with solution of sodium hypobromite was used to determine the location of spots after chromatography, in which barbiturates and urea appeared as yellow or white spot on a brown background, but these showed low sensitivity and gave a negative test in ureide. All metabolites can be detected by treatment of the sheet with 5% $KMnO_4$ solution followed by thorough washing with water. The sensitivity of the test permitted the detection of 5~10 γ of these compounds, and urea gave negative reaction in this case.

Better spraying reagent was the joint use of 1% $NaIO_4$ solution with 1% $KMnO_4$. The located chromatogram was revealed vividly and the sensitivity was somewhat higher than that of $KMnO_4$ alone.

Cooper and Brodie[9] investigated the *in vitro* metabolism of MHB by rabbit liver and identified its products by paper chromatography using butanol saturated with 1% ammonium hydroxide as a solvent. From this experiment, they concluded that the oxidation products were one or both of the keto-MHB, since keto-MHB (I) and (II) had the same Rf values. Bush, *et al.*[14] previously also obtained the same metabolites from the urine of dogs receiving MHB and suggested that these were 3- and 6-keto-MHB.

Previously we isolated α- and β-3-OH-MHB as the new oxidized products from the urine of rabbits receiving MHB and from a consideration of our experiments, reported[3] that it seems most reasonable to conclude that Bush's 6-keto-MHB is probably identical with our β-3-OH-MHB.

Similar to the method of Cooper and Brodie,[9] butanol saturated with 0.5N(0.86%) ammonium hydroxide was used as the solvent but 3-keto-MHB and 3-OH-MHB showed aproximate Rf values and these were not clearly separated by this technique (Fig. 1). Tochino[7] adopted the solvent system of butanol saturated with 5N ammonium hydroxide[4] in the detection of urinary metabolites of rabbits receiving MHB, and only gave a vague explanation. Our attempt to separate these compounds by the same solvent was also unsuccessful.

Finally, 3-OH-MHB, 3-keto-MHB, and other metabolites were separated on buffered filter paper, using butanol saturated with borate–NaOH buffer (pH 11) as the solvent system, and gave compact spots.

Bush, *et al.*[15] reported that only a trace of nor-MHB and unchanged MHB were excreted in the urine of dogs receiving MHB. Further, Deininger[15] demonstrated by

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paper chromatography that demethylation of MHB *in vivo* took place in mice. However, these two products were not detected in our experiment.

The quantitative investigation on the metabolites of MHB will be reported in the following paper.

Summary

Examination of urinary product of rabbits administered with MHB, by filter paper chromatography, indicated that ureide, 3-OH-MHB, 3-keto-MHB, 3-keto-nor-MHB, and unknown compounds (MHB-M (VI)) were excreted.

An excellent method for the separation of these compounds is the use of a buffered filter paper and butanol saturated with borate-NaOH buffer (pH 11).

For detection of the located metabolites, 1% solution of sodium periodate and 1% solution of potassium permanganate were used and ultraviolet spectrophotometry was adopted for the characterization of barbiturates.

(Received September 2, 1957)

UDC 547.673.1 : 544.621

5. Osamu Tanaka : Infrared Spectra of Anthraquinone Derivatives. I. The Effect of Hydroxyl, Acetoxyl, Methoxyl, and Methyl Substitutions on the C=O Stretching Frequency.

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Although some infrared spectral studies on anthraquinone derivatives were previously reported by Flett,[1] Josien *et al.*,[2,3] Wiles,[4] and some other workers,[5] more extensive studies are required to provide a precise information applicable to the chemistry of naturally occurring anthraquinones and their related compounds.

For this purpose, supplementing the previously published data, the present author carried out a measurement of infrared spectra of about 80 anthraquinone derivatives to examine the correlation of C=O stretching frequency to the substituents such as the hydroxyl, methoxyl, and methyl groupings.

It is well-known that change of the state of a sample sometimes causes a shift of C=O frequency, which must be ascribed to intermolecular forces. For quinoid compounds, Josien and her co-workers[2] observed that an anomalous diminution in C=O frequency sometimes occurs in the condensed phase compared to that in non-polar solvents such as carbon tetrachloride and carbon disulfide. Therefore, the measurement of infrared spectra in the solid state is not suitable for the present purpose. However, carbon tetrachloride and carbon disulfide are not suitable as a solvent, since anthraquinones in general, especially their hydroxyl derivatives, are very sparingly soluble in these solvents.

The effect of dioxane as a solvent for infrared spectral measurement has been

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