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.

(Received June 4, 1956)

U. D. C. 615.782.54-092.21

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.

(*Pharmaceutical Institute, Medical Faculty, University of Kyushu**)

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.

* Katakasu, Fukuoka (塚元久雄, 吉村英敏, 土岐 智).

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).

A great many investigations[3] on the ultraviolet absorption spectra of barbiturates have hitherto been reported and several studies[4] were also reported on the infrared absorption and these procedures were applied to this study.

Materials and Methods

Materials*—MHB (m.p. 142~143°) was supplied by Dainippon Seiyaku Co., Ltd. and nor-MHB (m.p. 209~211°(decomp.)) was prepared by the hydrolysis with 5% HCl of 5-cyclohexenyl-5-methyl-4-iminobarbituric acid supplied by the same company. 3-Keto-MHB (m.p. 160~161°),** 6-keto-MHB (m.p. 240~241°(decomp.)), and 3-keto-nor-MHB (m.p. 215~216°(decomp.)), were prepared by the oxidation[1] of MHB and nor-MHB, respectively, and cyclohexenylmethyl-N-methylacetylurea** was prepared by the decomposition[2] of a solution of MHB-Na. 3-OH-MHB[2](m.p. 213~215°(decomp.)) was obtained from the urine of rabbits receiving MHB.

Methods—The ultraviolet absorption spectra were measured by the Shimadzu photoelectric spectrophotometer with standard 10-mm. square quartz absorption cells. Borate-NaOH buffer (pH 11) used for the solvent was prepared as follows: To a solution of 12.404 g. of H_3BO_3 in 100 cc. of *N* NaOH solution, water was added to make 1 L. and further 1 L. of 0.1*N* NaOH solution was added to it. The concentration of the solution was 10 γ/cc. in all cases. In 0.5*N* NaOH solution, the absorption spectra of 5,5,N-trisubstituted barbituric acids must be measured as rapidly as posible, because they are easily decomposed in this medium.

The infrared absorption spectra were measured by the kind cooperation of Sankyo Co., Ltd.

Results and Discussion

Ultraviolet Absorption Spectra

In general,[3] the absorption peak of N-nonsubstituted 5,5-dialkylbarbituric acid appears at about 240 mμ in weakly alkaline solution, in which this substance forms a monopolar ion, and the peak shifts to a longer wave length (about 255 mμ) in a strongly alkaline solution because of the loss of the second proton with formation of a dipolar ion. On the other hand, the peak (about 245 mμ) in the 5,5,N-trisubstituted barbituric acid does not shift significantly even though the pH is raised, because only one dissociable proton is present.

In this experiment, nor-MHB and 3-keto-nor-MHB exhibited typical absorption peak of 5,5-disubstituted barbituric acid, whereas the ultraviolet absorption spectra of 3-keto-MHB, 6-keto-MHB, and 3-OH-MHB indicated these substances to be 5,5,N-trisubstituted barbituric acid. MHB-M (I) did not exhibit a characteristic absorption peak of barbiturate and this seemed to suggest that the barbituric acid ring had been destroyed.

Furthermore, in alcoholic solution, as a rule,[3] the barbiturates exhibit no absorption peak because of their non-disosciated form and even the cyclohexenyl group contributes little to ultraviolet absorption. In these observations, none of MHB, nor-MHB, and 3-OH-MHB possessed any absorption peak, but 3-keto-MHB, 6-keto-MHB, and 3-keto-nor-MHB, in which a carbonyl group had been introduced, exhibited a peak at about 220 mμ. It is presumed that this peak is due to α,β-unsaturated ketone although it was considerably different from the value calculated according to Woodward's rules[5] and such an abnormal absorption behavior would be caused by a barbituric

* All melting points are uncorrected.

** These compounds indicated the complete identity of their absorption spectra with MHB-M (II) and (I), respectively.[2]

3) cf. (a) L. R. Goldbaum: Anal. Chem., **24**, 1605(1952); (b) M. T. Bush, T. C. Butler, H. L. Dickison: J. Pharmacol. Exptl. Therap., **108**, 104(1953).

4) cf. C. J. Umberger, G. Adams: Anal. Chem., **24**, 1309(1952); E. W. Maynert, J. M. Dawson: J. Biol. Chem., **195**, 389(1952).

5) R. W. Woodward: J. Am. Chem. Soc., **64**, 76(1942).

acid ring adjacent to cyclohexenonyl group. Examples of abnormal absorption have been illustrated in N-heterocyclic compounds by Georgian,[6] and Marchant and Pinder.[7]

The evidence that these compounds are α,β-unsaturated ketones was also shown in the absorption peak at about 380 mμ of their 2, 4-dinitrophenylhydrazone in ethanolic solution. Furthermore, the difference[3b] between the absorption curves of keto compound and the corresponding parent substance was the greatest at about 230 mμ and this behavior also suggests that the compounds might be α,β-unsaturated ketones.

As mentioned by Bush, *et al.*[3b] introduction of a carbonyl group to the cyclohexenyl was associated with a profound increase in the ultraviolet absorption, both in the undissociated and ionic forms. However, absorbance of 3-keto-MHB in 0.5*N* NaOH solution increased not significantly probably because it was liable to decompose.

The spectra of the compounds mentioned above in 0.5*N* NaOH, borate-NaOH buffer (pH 11), and ethanolic solution are shown in Figs. 1~3, and their absorption maxima and molecular extinction coefficients at the maximum are listed in Table I.

Infrared Absorption Spectra

As shown in Fig. 4, the infrared absorption spectra of MHB-M (II), which was obtained from the urine of rabbits receiving MHB, and 3-keto-MHB, which was

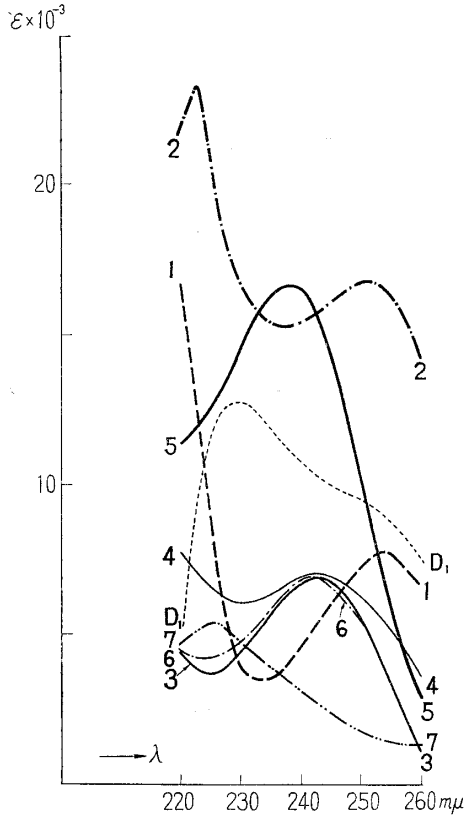

Fig. 1. Ultraviolet Absorption Spectra in 0.5*N* NaOH Solution

1 : Nor-MHB 2 : 3-Keto-nor-MHB
3 : MHB 4 : 3-Keto-MHB
5 : 6-Keto-MHB 6 : 3-OH-MHB
7 : MHB-M (I)
D_1 : Difference curve between 3-keto-nor-MHB and nor-MHB

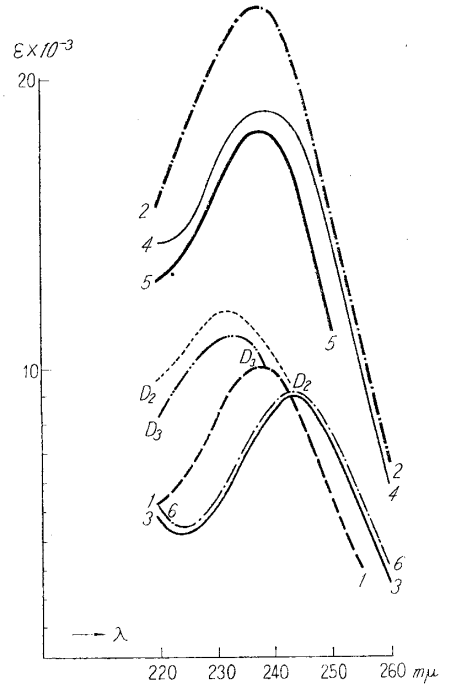

Fig. 2. Ultraviolet Absorption Spectra in Borate-NaOH Buffer (pH 11) Solution

1 : Nor-MHB 2 : 3-Keto-nor-MHB
3 : MHB 4 : 3-Keto-MHB
5 : 6-Keto-MHB 6 : 3-OH-MHB
D_2 : Difference curve between 3-keto-MHB and MHB
D_3 : Difference curve between 6-keto-MHB and MHB

6) V. Georginan : Chemistry & Industry, **1954**, 930.
7) A. Marchant, A. R. Pinder : J. Chem. Soc., **1956**, 327.

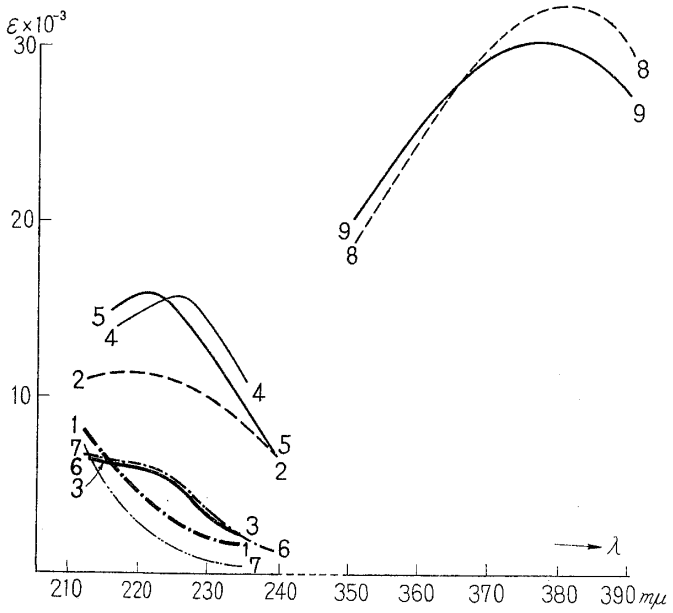

Fig. 3.
Ultraviolet Absorption Spectra in Ethanolic Solution

1 : Nor-MHB
2 : 3-Keto-nor-MHB
3 : MHB
4 : 3-Keto-MHB
5 : 6-Keto-MHB
6 : 3-OH-MHB
7 : MHB-M (I)
8 : 3-Keto-nor-MHB 2,4-dinitrophenylhydrazone
9 : 3-Keto-MHB 2,4-dinitrophenylhydrazone

TABLE I. Ultraviolet Absorption Maxima and Molecular Extinction Coefficients at the Maximum

	0.5 *N* NaOH			Borate-NaOH buffer			95% EtOH		
	λ_{min}	λ_{max}	ε_{max}	λ_{min}	λ_{max}	ε_{max}	λ_{min}	λ_{max}	ε_{max}
Nor-MHB	233	254	7,730	non	238	9,890	non	non	non
3-Keto-nor-MHB	237	223 251	23,240 16,760	〃	238	22,430	〃	218	11,430
MHB	225	242	6,880	224	244	9,000	〃	non	non
3-Keto-MHB	229	243	7,000	non	240	18,880	〃	225	16,000
6-Keto-MHB	non	239	16,730	〃	238	18,230	〃	221	16,000
3-OH-MHB	225	242	6,880	〃	244	9,180	〃	non	non
MHB-M (I)	non	225	5,380	—	—	—	〃	〃	〃
3-Keto-nor-MHB-DNPH*	—	—	—	—	—	—	〃	379	32,710
3-Keto-MHB-DNPH*	—	—	—	—	—	—	〃	376	30,650

* DNPH = 2, 4-Dinitrophenylhydrazone.

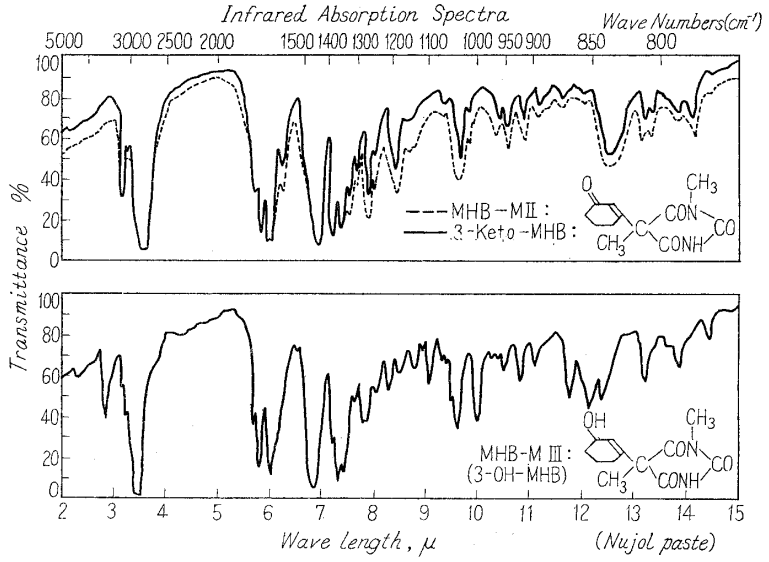

Fig. 4.

obtained by the chromic oxidation of MHB, were completely identical and the two absorption bands at 5.96 μ ($\nu_{C=O}$) and 6.19 μ ($\nu_{C=C}$) suggested the existence of α,β-unsaturated ketone in their structure.

MHB-M (Ⅲ), which was also isolated from the urine of rabbits receiving MHB, possessed a considerably sharp band at 2.87 μ and this suggested the existence of a hydroxyl group in its structure.

The authors are indebted to Sankyo Co. Ltd. for the measurement of the infrared sbsorption spectra and also to Dainippon Seiyaku Co. Ltd. for their supply of materials.

Summary

The ultraviolet absorption spectra of the metabolites from the urine of rabbits receiving MHB, the oxidation products of MHB and nor-MHB with chromium trioxide were measured, and the relation between these compounds and their structures was discussed.

The infrared absorption spectra of 3-keto-MHB and MHB-M (Ⅱ) from the previous work in this series indicated that the two are completely identical and would be α,β-unsaturated ketones and that of 3-OH-MHB suggested the existence of a hydroxyl group in the structure.

(Received June 4, 1956)

U. D. C. 547.854.4'861.2 : 582.284

74. Toru Masuda : Application of Chromatography. XXIX.[1] G Compound isolated from the Mycelium of *Eremothecium ashbyii*.

(*Research Laboratory, Takeda Pharmaceutical Industries, Ltd.**)

The components of the mycelium collected at various stages of the culture of *Eremothecium ashbyii* were studied by paper partition chromatography, and a green (G compound) and a purple (V compound) fluorescent bands were detected on the chromatograms of samples collected at a comparatively early stage. The former, which was already reported in a communication,[2] attracted a keen interest of the author because it seemed to have a close relation with the formation of riboflavin in the mycelium.

In the present paper the extraction, isolation, and purification of the substance, which were touched on in the communication, are described in more detail and further discussion is made on its structure.

Experimental

Isolation of G Compound—1) Two hundred grams of the wet mycelium obtained by 80-hr. culture of *Er. ashbyii* was extracted 3 times with a 500 cc. portion of water at 80° for 15 mins. and the combined extract was concentrated to 500 cc. The separated riboflavin was removed, 250 g. of $(NH_4)_2SO_4$ was added to the solution, and the resulting brown substance was filtered off. The filtrate was shaken 3 times with a 30-cc. portion of phenol and the phenol extract was shaken again with 500 cc. of ether and 30 cc. of water to transfer all flavine compounds into the water layer. The last operation was repeated twice and the combined aqueous solution was subjected to ad-

* Juso-Nishino-cho, Higashiyodogawa-ku, Osaka (増田 亨).

1) This constitutes a part of a series entitled "Application of Chromatography" by Satoru Kuwada. Part XXVIII. This Bulletin **3**, 434 (1955).

2) T. Masuda : This Bulletin **4**, 71 (1956).