to the resultant moments of $2\mu_0$ and $2\mu_0 \cos\frac{1}{2}\alpha = 1.15\mu_0$, respectively. The $N\begin{smallmatrix}C\\C\\C\end{smallmatrix}$ group moment $\mu_0$ can be approximated with fair accuracy by the moment of quinolizidine or that of hexahydrojulolidine. These moments were measured under the same condition as 0.74 D and 0.76 D, respectively. Therefore, it is indubitable that allomatridine has the conformation (I), while matridine corresponds to one of the three possible conformations (II), (III), and (IV).

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October 28, 1957

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*UDC 615.782.54-092.21*

## Detection of Hydroxyl Derivatives from the Urine of Men administered Ethylhexabital and Methylhexabital by Buffered Paper Chromatography.

It was previously 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),[1,2] and that after administration of MHB (methylhexabital, 5-cyclohexenyl-3,5-dimethylbarbituric acid) to rabbits, 3-OH-MHB (5-(3-hydroxy-1-cyclohexenyl)-3,5-dimethylbarbituric acid), 3-keto-MHB (5-(3-oxo-1-cyclohexenyl)-3,5-dimethylbarbituric acid), and three additional products were isolated from the urine.[3,4] Fretwurst, *et al.*[5] investigated the metabolic fate of EHB in the urine of men, and isolated a metabolite which was believed to be 3-keto-EHB. From these results we had postulated that hydroxylation of cyclohexenyl ring of MHB occurred owing to its structural difference with EHB.

Paper chromatography was used for the separation of these original drugs and metabolites. Hitherto, butanol saturated with ammonium hydroxide as the solvent system[6] were widely used for the paper chromatography of barbiturates and their metabolites. In the case of MHB, we failed to separate 3-OH-MHB and 3-keto-MHB from each other by this system,[6] since both gave almost similar Rf values. Later, it was learned that 3-OH-MHB was detectable by buffered paper chromatography, devised in our laboratory.[7]

In the present investigation, we applied this method for the detection of metabolites of EHB and MHB in human urine. As was expected, 3-OH-EHB was discovered and identified as another new metabolite of EHB, together with 3-keto-EHB and

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5) F. Fretwurst, J. Halberkann, F. Reiche : Münch. med. Wochschr., **79**, 1429(1932).
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7) H. Tsukamoto, H. Yoshimura, S. Toki : This Bulletin, **6**, in press.

unchanged material. Previously, however, it appeared that 3-keto-EHB was the only metabolite in the urinary extract of rabbits by paper chromatographic study of metabolites using butanol saturated with 5 *N* ammonium hydroxide as a solvent.[1] Moreover, we found the same metabolites of MHB, 3-OH-MHB, and 3-keto-MHB in rabbit urine.[3,4]

EHB was administered orally to men in a dose of 200 mg. The 8-hour urine was collected and extracted continuously with ethyl acetate at pH 4 for 15 hours. The residue left after evaporation of ethyl acetate was treated as previously reported.[7] The methanol solution of the sample was applied to a buffered filter paper (Toyo Roshi No. 50) which was completely impregnated with borate-NaOH buffer (pH 11), air dried, and subjected to ascending chromatography for 16~18 hours using butanol saturated with borate-NaOH (1:1) buffer (pH 11). Authentic samples of 3-keto-EHB, 3-OH-EHB,* and EHB were chromatographed simultaneously. The paper was dried in air, then sprayed with 1% aqueous solution of $NaIO_4$ and 1% aqueous solution of $KMnO_4$.[7,8] The three spots corresponding to 3-keto-EHB, 3-OH-EHB, and unchanged material were found. The ultraviolet spectra[9] of these products and authentic samples were identical and exhibited characteristic peaks at 239 mμ in borate-NaOH buffer (pH 11) solution.

MHB was treated by the same procedure as above, and the presence of 3-keto-MHB, 3-OH-MHB, and ureide (1-(2-cyclohexenylpropionoyl)-3-methyl urea), was confirmed.

Later, in connection with these results, a very small amount of 3-OH-EHB was isolated from the urine of rabbits receiving EHB.

Further details of these experiments will be reported in the near furture.

The writers are indebted to Miss H. Ōta for technical co-operation, and also to Shionogi & Co., Ltd. and Dainippon Pharmaceutical Co., Ltd. for their supply of the materials used in this work.

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October 30, 1957

* 3-OH-EHB was synthesized by the Meerwein-Ponndorf reduction of 3-keto-EHB.

8) M.L. Wolfrom, J.B. Miller: Anal. Chem., **28**, 1037(1956).
9) H. Tsukamoto, E. Takabatake, T. Ariyoshi: This Bulletin, **3**, 459(1955).