UDC 615.78-092.21

85. Akira Yamamoto, Hidetoshi Yoshimura, and Hisao Tsukamoto: Metabolism of Drugs. XXIX.*1 Metabolic Fate of Meprobamate. (2). Further Studies on the Structure of the Metabolites.

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In the previous investigation*1 of this series, six metabolites in the urine of rabbits and dogs administered with meprobamate have been examined. They were keto-(I), hydroxy-(II), carboxy-meprobamate (III), and three glucuronides. Of the three glucuronides, one (IV) was demonstrated to be a new type of N-glucuronide of meprobamate and the other (V) the ether-type O-glucuronide of hydroxy-meprobamate, but the structure of the third glucuronide (VI) remained still unclarified, since only a small amount of (VI) was isolated.

As already discussed in the preceding paper, the findings of Walkenstein, *et al.*[1] that the major metabolite of meprobamate in dogs was hydroxy-meprobamate(2-hydroxymethyl-2-propyl-1,3-propanediol dicarbamate), did not seem to be correct and the authors proposed that it should be 2-methyl-2-(2-hydroxypropyl)-1,3-propanediol dicarbamate.

In the present investigation, additional evidences about the structures of the oxidation products of meprobamate *in vivo* are presented.

First of all, the synthesis of hydroxy-meprobamate (II) was undertaken in order to justify the foregoing discussion. According to the method of Maynert, *et al.*[2] who synthesized 5-ethyl-5-(3-hydroxybutyl)barbituric acid from 5-ethyl-5-(3-butenyl)barbituric acid by hydration with conc. sulfuric acid, 2-methyl-2-allyl-1,3-propanediol dicarbamate (VII) was chosen as the starting material.

The result was, however, not very satisfactory since only a small amount of the expected hydroxy-meprobamate (2-methyl-2-(2-hydroxypropyl)-1,3-propanediol dicarbamate) was obtained after alumina chromatography. This product, although it failed to crystallize, gave a positive ceric nitrate test and an iodoform reaction. When it was compared paper chromatographically with hydroxylated metabolite (II), it showed the same Rf value.

Besides this product, an unexpected crystalline compound, m.p. 62~63°, (VIII) was also isolated from the reaction mixture, and it turned out to be a key compound.

Next, an attempt was made to convert (II) into (VII) by means of halogenation of (II) followed by dehydrohalogenation with thionyl chloride and pyridine. Of great interest was the fact that the major product, m.p. 62~63°, was not the expected compound (VII), but was identical with the compound (VIII) obtained from (VII) by treatment with conc. sulfuric acid. The identity of both compounds was confirmed by mixed melting point test and comparison of infrared spectra. Elemental analysis of this compound (VIII), m.p. 62~63°, was consistent with the formula, $C_8H_{15}O_3N$, which corresponded to the monocarbamate instead of the dicarbamate as calculated from its nitrogen content. Hence it was simply considered that this might be 2-methyl-2-allyl-1,3-propanediol monocarbamate produced by partial hydrolysis of (VII).

However, this assumpiton became soon improbable and it was finally concluded from

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*1 Part XXVIII. This Bulletin, **10**, 522 (1962).

*2 Katakasu, Fukuoka (山本 陽, 吉村英敏, 塚元久雄).

1) S. S. Walkenstein, C. M. Knebel, *et al.*: J. Pharmacol. Exptl. Therap., **123**, 254 (1958).

2) E. W. Maynert: J. Biol. Chem., **195**, 403 (1952).

the following facts that the structure of (Ⅷ) should be of the type of a cyclization compound such as a tetrahydrofuran derivative. 1) No reaction occurred on treatment of (Ⅷ) with phosgen and ammonia in the preparation of the dicarbamate. 2) (Ⅷ) gave a negative color reaction for alcohol. 3) (Ⅷ) did not decolorize bromine in acetic acid. 4) No reaction occurred on catalytic reduction over palladium-carbon. 5) Infrared spectrum of (Ⅷ) did not show any absorption of a vinyl group as shown in Fig. 1.

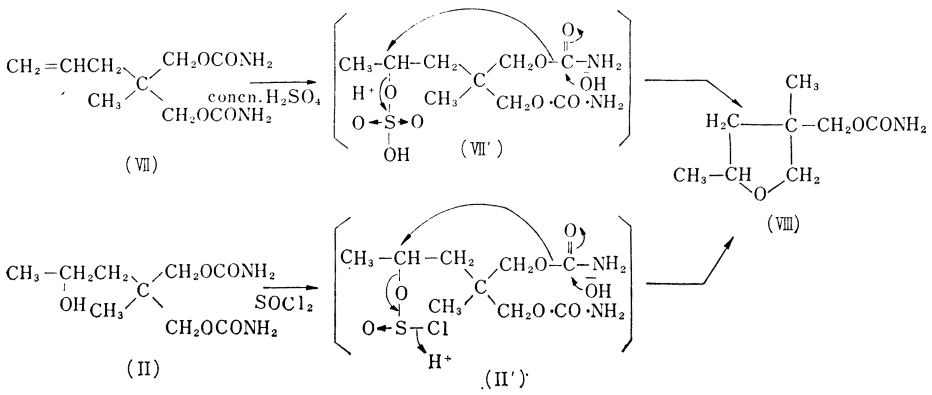

Chart 1.

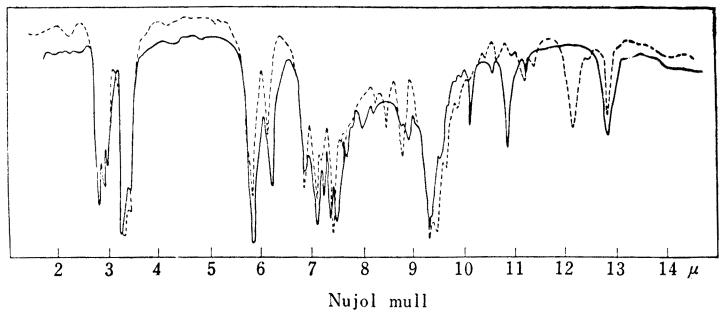

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Fig. 1. Infrared Spectra of Compound (Ⅶ) and (Ⅷ)

—— (Ⅶ) ····· (Ⅷ)

The structure of (Ⅷ) might be derived from intramolecular nucleophilic attack of the oxygen atom on the intermediates (Ⅶ′) and (Ⅱ′) as shown in Chart 1.

Barrow, *et al.*[3] found in the infrared spectra of cyclic ethers that the principal characteristics of tetrahydrofuran spectra were the strong bands appearing at 9.1 to 9.3 and 11 μ, and that the strong 11 μ band was splitted into several bands in the case of the substituted dimethyl compounds.

In the study of dihydrothiamine, Hirano, *et al.*[4] found that the compound possessing a perhydrofurothiazole ring and all the compounds of this series exhibited a specific absorption between 11.9 and 12.1 μ due to the tetrahydrofuran ring.

3) G. M. Barrow, S. Searles: J. Am. Chem. Soc., **75**, 1175 (1953).
4) H. Hirano, T. Iwazu, S. Yurugi: Yakugaku Zasshi, **77**, 241 (1957).

Since our compound (VIII) has specific absorptions at 9.5, 11.2, and 12.1 μ, it was considered to have a tetrahydrofuran ring.

From these facts it seems very likely that (VIII) is 3,5-dimethyl-3-tetrahydrofuryl methyl carbamate.

Aside from the reaction mechanism and the correct structure of the reaction product in this case, the formation of this compound (VIII) from both of (VII) and (II) proved conclusively that the hydroxyl group was located on the propyl group, not on the methyl group. Accordingly, the structure of (II) was confirmed to be 2-methyl-2-(2-hydroxypropyl)-1,3-propanediol dicarbamate.

Since keto-meprobamate (I) was found to be identical with the oxidation product of (II) and was reduced by lithium alminium hydride to give (II), the structure of this ketone was also confirmed to be 2-methyl-2-(2-oxo-propyl)-1,3-propanediol dicarbamate.

As mentioned in the previous paper, the analysis, infrared spectrum, formation of methyl ester, and neutralization equivalent of carboxy-meprobamate (III) suggested that a terminal methyl of the propyl group had been converted to a carboxylic acid group. This assumption was also supported by the paper chromatographic and paper electrophoretic behaviors.

In order to clarify which alkyl radical of meprobamate was oxidized, the degradation of (III) was attempted to obtain either butane-1,3,3-tricarboxylic acid or propyl malonic acid as shown in Chart 2. As a result, it was found that the degradation product should have the former structure by comparison with an authentic sample synthesized as shown in Chart 2. Therefore, it can be stated definitely that carboxy-meprobamate is 2-methyl-2-(2-carboxyethyl)-1,3-propanediol dicarbamate.

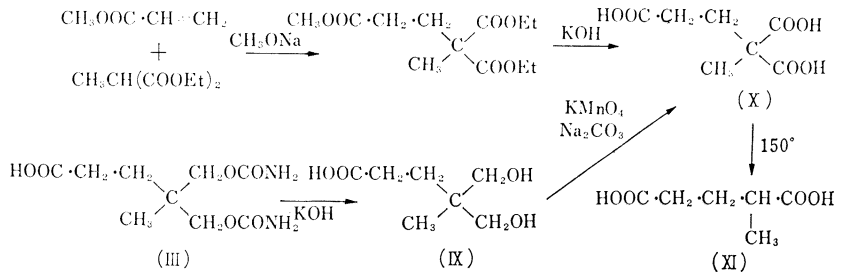

Chart 2.

Experimental

2-Methyl-2-allyl-1,3-propanediol——A solution of 5 g. of $LiAlH_4$ in 20 cc. of Et_2O was placed in a flask equipped with a reflux condenser, a dropping funnel and a mechanical stirrer and the system was protected from moisture by $CaCl_2$ tubes. Through the dropping funnel, 20 g. of methylallylmalonic diethyl ester (b.p. 222~226°) in 50 cc. of Et_2O was introduced at such a rate that Et_2O was gently refluxed. After the addition was complete, the mixture was refluxed for 1.5 hr. with continued stirring, and then cooled in an ice-salt mixture. The excess hydride was destroyed by dropwise addition of H_2O with cautious cooling of the flask, and the solution was acidified with dil. H_2SO_4. After separation of the Et_2O layer, the aqueous layer was extracted continuously with Et_2O for 15 hr. The product obtained after evaporation of the Et_2O from the dried Et_2O extracts was fractionally distilled, and 7 g. of 2-methyl-2-allyl-1,3-propanediol ($b.p_5$ 119~125°) was obtained.

2-Methyl-2-allyl-1,3-propanediol Dicarbamate (VII)——The synthesis of dicarbamate was carried out according to the method of Ludwig, *et al.*[5] To a solution of 7.5 g. of $COCl_2$ in 8 cc. of toluene

5) B. J. Ludwig, E. C. Piech : J. Am. Chem. Soc., **73**, 5779 (1951).

cooled at −10°, a cooled solution of 5 g. of 2-methyl-2-allyl-1,3-propanediol and 14 g. of antipyrine in 40 cc. of $CHCl_3$ was added with stirring at such a rate that the temperature of the mixture was maintained at −5 to 0°. The mixture was allowed to warm slowly to room temperature and was kept at this temperature overnight. The seperated antipyrine hydrochloride was removed and the chlorocarbonate converted directly to the amide by treating the filtrate with gaseous NH_3 with moderate cooling. The amide was separated by filtration, freed from NH_4Cl by washing with cold H_2O and recrystallized from H_2O. 5.9 g. of 2-methyl-2-allyl-1,3-propanediol dicarbamate, m.p. 116°, was obtained. *Anal.* Calcd. for $C_9H_{16}O_4N_2$: C, 50.00; H, 7.4; N, 12.96. Found: C, 50.14; H, 7.57; N, 12.71.

Reaction of (VII) with Sulfuric Acid[2]——A solution of 3 g. of (VII) in 9 cc. of 75% H_2SO_4 was allowed to stand at 10° to 15° for 48 hr. The dark orange liquid was poured over 40 g. of cracked ice. The solution was adjusted to pH 6 and extracted 5 times with 100 cc. portions of AcOEt. The AcOEt extracts were combined, washed, dried and evaporated to yield a partially crystalline oily substance. The crystals were separated to form colorless needles, m.p. 180°. The structure of this product remained undetermined.

The remaining oily substance was separated into three fractions giving a positive Ehrlich's reaction by chromatography through an alumina column. The first effluent with benzene-AcOEt (1:1) was evaporated and recrystallized from benzene-cyclohexane to colorless prisms, m.p. 62~63° (VIII). *Anal.* Calcd. for $C_8H_{15}O_3N$: C, 55.49; H, 8.67; N, 8.09. Found: C, 55.61; H, 8.70; N, 8.32. The second with AcOEt gave the compound (VII). The last with Me_2CO which showed positive iodoform and ceric nitrate tests, was found to be identical with the Metabolite (II) paper chromatogra phycally, but this substance failed to crystallize.

Reaction of Hydroxy-Meprobamate (II) with Thionyl Chloride and Pyridine——To a mixture of 0.3 g. of (II) and 0.13 g. of pyridine, 0.2 g. of $SOCl_2$ was added with ice cooling. The mixture was allowed to stand at room temperature for 1 hr. and then warmed at 55° for another 1 hr. To this reaction mixture, 20 cc. of AcOEt was added and the solution was washed with H_2O. The washings were extracted 3 times with 20 cc.-portions of AcOEt. The combined AcOEt extracts were again washed with H_2O, dried, evaporated to dryness *in vacuo*. The residual oily substance was chromatographed through an alumina column. The effluent with benzene-AcOEt (1:1) gave crystals, m.p. 60~62°, which were recrystallized from benzene-cyclohexane to colorless prisms, m.p. 62~63° (VIII). *Anal.* Calcd. for $C_8H_{15}O_3N$: C, 55.49; H, 8.67; N, 8.09. Found: C, 55.66; H, 8.71; N, 8.07.

This substance (VIII) was also obtained with $SOCl_2$ in the absence of pyridine, and with conc. H_2SO_4. No H_2 uptake occurred on catalytic reduction of (VIII) over Pd-C, resulting in recovery of the starting material.

Butane-1,3,3-Tricarboxylic Acid (X)——This was synthesized from methyl acrylate and diethyl methylmalonate by Michael condensation followed by hydrolysis according to the method of Swan.[6] Needles, m.p. 134° (decomp.) from Me_2CO-$CHCl_3$. *Anal.* Calcd. for $C_7H_{10}O_6$: C, 44.2; H, 5.25. Found: C, 44.07; H, 5.31.

α-Methylglutaric Acid (XI)——This was obtained by decarboxylation of tricarboxylic acid (X). Decarboxylation was completed by heating in an oil bath at 150°. Needles, m.p. 77° from H_2O. *Anal.* Calcd. for $C_6H_{10}O_4$: C, 49.23; H, 6.85. Found: C, 49.41; H, 6.94.

Hydrolysis of Carboxy-Meprobamate (III)——A solution of 0.7 g. of (III) in 15 cc. of 15% KOH solution was refluxed in an oil bath for 2 hr. The mixture was adjusted to pH 2.5 with dil. H_2SO_4 and extracted continuously with Et_2O for 10 hr. to give 0.36 g. of diol (IX), which was submitted to the next oxidation reaction without further purification.

Oxidation of Diol (IX)——To a solution of 0.36 g. of the diol in 100 cc. of H_2O, 1.2 g. of $KMnO_4$ and 1.0 g. of anhyd. Na_2CO_3 were added. This mixture was shaken thoroughly, stoppered, and allowed to stand at room temperature for 1 week. The excess of $KMnO_4$ was destroyed by addition of $NaHSO_3$, the mixture was then acidified to pH 2.5, and extracted 3 times with 100 cc.-portions of AcOEt. The combined extracts were washed, dried, and evaporated to dryness. The colorless crystalline residue obtained, was recrystallized from Me_2CO-$CHCl_3$ to needles, m.p. 133~134° (decomp.). (yield, 0.23 g.). *Anal.* Calcd. for $C_7H_{10}O_6$: C, 44.20; H, 5.25. Found: C, 44.07; H, 5.32.

This material was confirmed to be identical with butane-1,3,3-tricarboxylic acid (X) by admixture and infrared comparison.

Furthermore, decarboxylation of this compound yielded needles, m.p. 70~74°. This was confirmed to be identical with α-methylglutaric acid (XI).

The authors are indebted to Messrs. E. Tamura and K. Nishiyama for technical assistance, to Mr. H. Yano for the determination of infrared spectra, and to Miss S. Tada for the elementary analyses. Thanks are also due to Daiichi Seiyaku Co., Ltd. for their supply of meprobamate.

6) G. A. Swan: J. Chem. Soc., **1955**, 1039.

Summary

The structures of three metabolites isolated from the urine of rabbits and dogs admintstered with meprobamate (2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate) were established as keto-meprobamate [2-methyl-2-(2-oxo-propyl)-1,3-propanediol dicarbamate], hydroxy-meprobamate [2-methyl-2-(2-hydroxypropyl)-1,3-propanediol dicarbamate] and carboxy-meprobamate [2-methyl-2-(2-carboxyethyl)-1,3-propanediol dicarbamate].

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86. Tsutomu Momose and Masaru Nakamura : Organic Analysis. XXXIII.*1 Mechanism of the Color Reaction between Fructose and N-Methyldiphenylamine.

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N-Methyldiphenylamine gives a sensitive color reaction with fructose when heated in a mixture of hydrochloric acid and ethanol.[1] This reaction can successfully be used in the detection and estimation of the sugar in the presence of glucose, but the mechanism of the reaction has remained unknown. This paper describes a probable mechanism of the reaction, isolating the main dye in crystalline form.

Isolation of the Dye——When a definite amount of fructose was refluxed with varied amounts of N-methyldiphenylamine in a mixture of hydrochloric acid and ethanol, the intensity of the developed violet color increased with the increasing amount of the reagent. One mole of fructose needed two moles of N-methyldiphenylamine to give the maximum intensity (Fig. 1). But the isolation of the dye was carried out from the reaction mixture in which fructose was excess than the calculated amount.

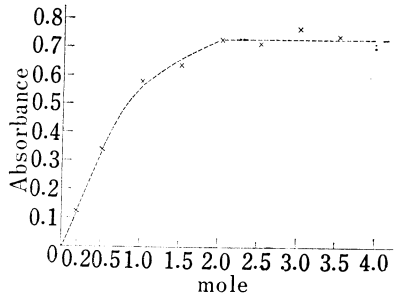

Fig. 1. Mole Ratio of N-methyldihenylamine to Fructose

540 mg. of fructose and varied amount of N-methyldiphenylamine were dissolved in a mixture of conc. HCl 30 ml.+EtOH 30 ml., refluxed for 5 min., diluted to 2500 times, and measured at 629 mμ.

To the developed mixture, a small amount of water was added to separate resinous substances. After a short time the precipitate was removed, a large amount of water was added to the filtrate, and allowed to stand overnight. The separated crystalline dye

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*1 Part. XXXII : Rinsho Kensa, **5** 451 (1961).
*2 Katakasu, Fukuoka (百瀬 勉, 中村 優).
1) G. Kallinich, H. Thies : Chem. Ber., **87**, 759 (1954).