185. Hisao Tsukamoto, Hidetoshi Yoshimura, and Kiyoshi Tatsumi:
Metabolism of Drugs. XXXIX.\textsuperscript{a1} Further Studies on Carbamate N-Glucuronide Formation in Animal Body.\textsuperscript{a2}

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During the course of study on metabolic fate of meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate), the authors\textsuperscript{1}) noticed that meprobamate conjugated with glucuronic acid was excreted in a considerable amounts together with other metabolites in the urine of rabbits after dosing with the drug. Ludwig, \textit{et al.}\textsuperscript{3}) also reported independently on the excretion of this characteristic metabolite in human beings. In the both laboratories, however, its N-glucuronide structure was deduced only from the consideration that meprobamate possessed nothing but NH\textsubscript{2} of carbamate for a functional group to form a glucuronide.

Further investigation in our laboratory\textsuperscript{5}) made possible to isolate this metabolite as amorphous powder and led to the conclusion that its structure seemed to be meprobamate N-mono-\(\beta\)-d-glucopyranosiduronic acid (I) mainly on the basis of the infrared absorption spectrum of its methyl acetyl derivative. It was also proved that acid-catalyzed condensation of meprobamate with glucuronic acid afforded the same compound as the metabolite and the glucuronide formation could be effected by uridine diphosphate glucuronic acid and glucuronol transferase using \textit{in vitro} system, but not by a spontaneous reaction during the isolation procedure.

Only problem remained in our hands at that time was the crystallization of this amorphous glucuronide and now it was solved by its conversion to sodium salt. The present paper will also describe the synthesis and the possibility of the urinary occurrence of other carbamate N-glucuronides.

The previous isolation procedure of meprobamate N-glucuronide from both the urine and synthetic route was rather tedious\textsuperscript{5}) and a little modification was made in this paper as follows: The glucuronides mixture obtained from both sources was purified through counter current distribution method and the resulting N-glucuronide fraction was then submitted to methylation and acetylation in usual manner. After silica gel chromatography of the reaction mixture, the methyl derivative was treated with a solution of an equivalent amount of sodium methoxide in methanol, yielding the crystalline material (II), m.p. 170~175°(decomp.). The infrared absorption spectrum of this compound exhibited, in addition to other characteristic peaks, a strong band due to carboxylic ion at 6.21\(\mu\) which well interpreted the structure of sodium meprobamate N-mono-\(\beta\)-d-glucopyranosiduronate (Fig. 1). The analyses were also in good agreement with the formula, \(\text{C}_{38}\text{H}_{32}\text{O}_{16}\text{N}_{2}\text{Na}\cdot\text{H}_{2}\text{O}\). The both metabolic and synthetic samples were entirely identical in details, and could be reconverted with dilute hydrochloric acid to the amorphous free form (I) which was isolated previously.

In order to reconfirm its pyranosiduronate structure, the periodate oxidation of II was carried out. It has been well established in our laboratory\textsuperscript{5}) that the rate of

\textsuperscript{a2} The preliminary communication of this paper was briefly reported in Life Sciences, No. 6, 382 (1963).
periodate oxidation of furanosiduronide type compound was extremely slow as compared with those of pyranosiduronide type, using about 2 moles equivalent of the oxidant, and the result obtained here showed clearly that II had the pyranosiduronide structure.

Next attention was directed to urethane, the simplest drug having a carbamate group, whether it could form N-glucuronide ornit. Preceding this study, the synthesis of the authentic sample was attempted in the following two ways. The first route was the utilization of methyl 1-deoxy-1-ethoxythiocarbonylamino-2,3,4,4-tri-O-acetyl-β-D-glucopyranosiduronate (III) which was unequivocally synthesized recently by Kuranari.\(^5\)

It was successfully desulfurized with silver nitrate to methyl 1-deoxy-1-ethoxycarbonylamino-2,3,4,4-tri-O-acetyl-β-D-glucopyranosiduronate (IV), m.p. 128~129°, [α]_D^25 +14.9°, which was, in turn, converted to sodium 1-deoxy-1-ethoxycarbonylamino-β-D-glucopyranosiduronate (V), by treatment with a solution of sodium methoxide in absolute methanol. Hydrolysis of V with dilute hydrochloric acid afforded urethane N-glucuronide(1-deoxy-1-ethoxycarbonylamino-β-D-glucopyranosiduronic acid) (VI), m.p. 154~156°.

The another route was the acid-catalyzed direct condensation of urethane with glucuronic acid, which was also adopted in the synthesis of meprobamate N-glucuronide. One mole each of urethane and glucuronic acid was condensed in 5% sulfuric acid or

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with Amberlite IR 120 to VI which was further converted to its methyl acetyl derivative (IV). It was easily led to the crystalline sodium salt (V) on its treatment with sodium methoxide solution.

Above fact not only shows that acid-catalyzed urethane N-glucuronide possesses β-pyranosiduronide structure, but also strongly suggests that the structure of meprobamate N-glucuronide obtained in similar manner should be also β-pyranosiduronide type.

The excretion of VI into the rabbit urine after dosing with urethane (300 mg./kg.) was examined by the paper chromatography, but no spot corresponding to VI was detected. This result is not so surprising because it is well known that carbamate linkage of urethane is almost completely broken down, only a very small amount being excreted unchanged in animal body. However, the drug having carbamate group and being excreted unchanged in a considerable amounts seems very likely to form N-glucuronide as one of its metabolites rather in common. Therefore another investigation has been undertaken on N-glucuronide formation of ethinamate (1-ethynylcyclohexyl-carbamate) which is also using as a hypnotic. Although several workers7–9) have already investigated on the metabolic fate of ethinamate, and described the occurrence of its O-glucuronide in the mammalian urine, no one has noticed about its N-glucuronide formation.

In the present study, the authors confirmed the excretion of its N-glucuronide into the urine of rabbits receiving the drug (300 mg./kg.) by means of paper chromatography, in which the Rf value of its N-glucuronide was higher (0.68) than those of its O-glucuronide in the solvent system of BuOH–AcOH–H2O (4:1:5) and this property was just same as of meprobamate N-glucuronide.10) Isolation of this metabolite from the rabbit urine was effected by its conversion to the crystalline sodium salt analogously as in meprobamate. On its hydrolysis with dilute hydrochloric acid, glucuronic acid could be detected on the paper chromatogram, but not ethinamate. This unexpected result could be interpreted by the fact that ethinamate was quite labile during acid treatment.10) The chemical synthesis of this metabolite through acid-catalyzed condensation will encounter to the same problem and so the authors have not a synthetic evidence leading to the structure at the present time, however the infrared spectrum (Fig. 2.) and the analytical data, in addition to other properties, it is very likely that the metabolite should be ethinamate N-β-d-glucopyranosiduronide (VII).

Meprobamate N-mono-β-d-glucopyranosiduronide acid revealed no anticonvulsant action even with high dose (2 g./kg.).

Experimental

Chemical Synthesis of Sodium Meprobamate N-Mono-β-d-glucopyranosiduronate (II)—A mixture of glucuronic acid (1 g.) and meprobamate (1.13 g.) in 5% H2SO4 (1 ml.) was heated on a water bath at 70° for 45 hr. with intermittent stirring. After an addition of 30 ml. of H2O to the reaction mixture, it was extracted with Et2O (50 ml. x 6) to remove the unreacted meprobamate and the aqueous solution was evaporated to dryness in vacuo. The residue was again dissolved in a small volume of MeOH, filtered, and the filtrate was evaporated to dryness. The resulting gum was subjected to countercurrent distribution between 50 ml. of BuOH and the same volume of H2O using eleven transfers. The contents of the fourth to the ninth, (540 mg.) which were paper chromatographically shown to consist mainly of meprobamate N-glucuronide, were combined, dissolved in a small volume of H2O, and the

9) T. Murata : This Bulletin, 8, 629 (1960); 9, 146, 167, 169, 334, 335 (1961).
solution was extracted first with Et,O (50 ml. × 4) and then with AcOEt (100 ml. × 8). The AcOEt extract was dried over anhyd. Na₂SO₄, evaporated to dryness in vacuo, and converted to methyl acetyl derivative by usual method. After purification through silica gel chromatography as described in the previous paper,³ the methyl acetyl derivative was dissolved in a mixture of abs. MeOH (10 ml.) and 2N MeONA solution (0.27 ml.). The solution was allowed to stand in a refrigerator for 2 days and evaporated to dryness in vacuo. The crystalline material precipitated from MeOH when the residue was rubbed with a glass rod. After keeping it in a refrigerator overnight it was recrystallized from aqu. liquor EtOH to colorless crystals, m.p. 170~175° (decomp.); [α]₂⁰ +21.6° (c = 2.08, H₂O). When the mother was submitted to alumina chromatography the same crystalline material was obtained a little amount from the eluate with 90% aqu. MeOH.

IR λ_max μ : 2.94 (νOH, νNL), 5.80~5.85 (νC-O), 6.21 (νCOO⁻, νNH), 6.45 (νNH). Anal. Calcd. for C₁₃H₁₅O₃N₂Na·H₂O : C, 41.47; H, 6.22; N, 4.65. Found : C, 41.42; H, 6.32; N, 6.25. Treatment of II with dil. HCl produced I as amorphous solid.

Isolation of II from the Rabbit Urine——The crude glucuronide gum obtained from the urine of rabbits receiving 17 g. of meprobamate according to the method described in the previous report,³ was dissolved in 20 ml. of H₂O and extracted with AcOEt (100 ml. × 10). The extract was dried over anhyd. Na₂SO₄, evaporated to dryness in vacuo and this crude glucuronide fraction was submitted to countercurrent distribution, followed by its conversion to the methyl acetyl derivative and then by the treatment with MeONA solution same as described in the synthetic procedure of I. Yield : 0.12 g. m.p. 170~175° (decomp.); [α]₂⁰ +21.5° (c = 2.09, H₂O). Anal. Calcd. for C₁₃H₁₅O₃N₂Na·H₂O : C, 41.47; H, 6.22; N, 4.65. Found : C, 41.64; H, 6.25; N, 6.46. The melting point of this compound was not depressed by admixture with synthetic (I) and the IR spectra of the samples from both sources were completely superimposable.

Chemical Synthesis of 1-Deoxy-1-ethoxy carbonylamino-β-D-glucopyranosiduronic Acid (VI) and its Derivatives

Method A——Synthesis of methyl 1-deoxy-1-ethoxycarbonylamino-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronic (IV) : To a hot solution of 1.6 g. of methyl 1-deoxy-1-ethoxythiocarbonylamino-2,3,4-tri-O-acetyl-β-D-glucopyranosiduronic (III) in 32 ml. of EtOH, a solution of 1.6 g. of AgNO₃ in 8 ml. of H₂O was added and heated at 50~55° on a water bath. After 5 min. 0.1N NaOH was added to the reaction mixture in order to neutralize HNO₃ liberated from AgNO₃. This promoted the coagulation of Ag₃S and helped to prevent HNO₃ from hydrolyzing acetyl groups of the sugar moiety. The solution was heated until Ag₃S coagulated almost completely (about 10 min.). It was then cooled and filtered. The filtrate was concentrated to dryness in vacuo. The residue was submitted to silica gel (100 g.) chromatography using 150 ml. of CHCl₃ as effluent solvent for the complete removal of Ag₃S. The UV spectrum of this effluent still exhibited a small peak at 245 mµ which meant a little contamination with unchanged III. Therefore, it was again treated with AgNO₃ in the similar manner as mentioned above. The reaction mixture was evaporated to dryness in vacuo, extracted with CHCl₃, and the extract was evaporated to dryness. The residue was crystallized from EtOH to colorless needles, m.p. 125~127°. Yield : 0.3 g. After repeated recrystallization from EtOH the melting point raised to 128~129°. [α]₂⁰ +14.0° (c = 1.68, CHCl₃). IR λ_max μ : 2.98 (νOH), 5.66~5.77 (νC=O), 6.45 (νNH). Anal. Calcd. for C₁₃H₁₅O₃N : C, 47.41; H, 5.68; N, 3.46. Found : C, 47.45; H, 5.86; N, 3.55.

Synthesis of sodium 1-deoxy-1-ethoxycarbonylamino-β-D-glucopyranosiduronic (V) : To a mixture of 30 ml. of abs. MeOH and 0.5 ml. of 2N MeONA solution, 0.3 g. of IV was added and the mixture was allowed to stand overnight in a refrigerator. The reaction mixture was evaporated to dryness in vacuo, leaving crystalline material. It was recrystallized from eq. EtOH to colorless crystals. Yield : 0.2 g. It did not show a definite melting point up to 250° and began to decompose from about 180°; [α]₂⁰ -25.0° (c = 2, H₂O). IR λ_max μ : 2.80~3.07 (νOH, νNH), 5.74 (νC=O), 6.26 (νCOO⁻, νC=O), 6.50 (νNH). Anal. Calcd. for C₁₃H₁₅O₃N : C, 37.63; H, 4.88; N, 4.88. Found : C, 37.54; H, 5.05; N, 4.96.

Synthesis of 1-deoxy-1-ethoxycarbonylamino-β-D-glucopyranosiduronic acid (VI) : To a solution of V in 0.5 ml. of H₂O, 0.13 ml. of 10% HCl was added. The mixture was concentrated to a small volume in vacuo and left overnight in a refrigerator. The crystalline material separated from mother liquor was collected and recrystallized from H₂O to colorless needles, m.p. 154~156°. [α]₂⁰ = -36.9° (c = 2.03, H₂O). Yield : 30 mg. IR λ_max μ : 2.75, 3.04 (νOH, νNH), 5.78~5.98 (νC=O), 6.34 (νNH). Anal. Calcd. for C₁₃H₁₅O₃N·1/2H₂O : C, 39.42; H, 5.84; N, 5.11. Found : C, 39.64; H, 5.81; N, 5.38.

Hydrolysis of this compound with 5% HCl at 75° for 1 hr. afforded crystalline urethane, and another component, glucuronic acid could be detected on the paper chromatogram.

Method B——Synthesis of IV and V : A mixture of glucuronic acid (1.5 g.), urethane (1 g.), Amberlite IR 120 (1.5 ml.), and H₂O (1.5 ml.) was heated at 75~80° for 27 hr. on a water bath with stirring. The resin was separated from the reaction mixture by filtration, and the filtrate was evaporated to dryness in vacuo. The residue was subjected to countercurrent distribution between BuOH and H₂O using 6 transfers. The third to the fifth were combined, dissolved in a small volume of H₂O, extracted a contaminated material with Et₂O, and the aqueous solution was evaporated to dryness in vacuo. The residue
was converted to methyl acetyl derivative by usual method and it was recrystallized from EtOH to colorless needles, m.p. 128°-129°. Anal. Calcd. for C₂₆H₄₃O₇N: C, 47.41; H, 5.68; N, 3.45. Found: C, 47.45; H, 5.88; N, 3.55. The melting point of this compound was not depressed by admixture with iv prepared by Method A. The IR spectra of both compounds were completely identical. V could be obtained through this compound by treatment with a solution of MeONa in abs. MeOH as described in Method A, and also directly by treatment of the acid-catalyzed reaction product purified through countercurrent distribution with NaOH solution.

Synthesis of VI: A mixture of glucuronic acid (1.1 g) and urethane (0.5 g) in 5% H₂SO₄ (1 ml) was heated at 70° for 5 hr. on a water bath with intermittent stirring and left for a long time at room temperature. The deposited crystalline material was separated from mother liquor, and recrystallized from H₂O to colorless needles, m.p. 154°-156°. Yield: 0.95 g. This compound was entirely identical with VI prepared in Method A by the mixed fusion test and the comparison of their IR absorption spectra.

Examination of Urethane N-Glucuronide Formation in Rabbit—A 24 hr. urine of nine rabbits after dosing totally with 6 g. of urethane (0.3 g./kg. body weight) was treated to obtain glucuronide gum by usual method. The resulting gum was examined whether it contained N-glucuronide or not by paper chromatography, but no result corresponding to the authentic sample (VI) was detected.

Isolation of Sodium 1-Ethenylcyclohexyl Carbamate N-β-D-Glucopyranosiduronate (VII) from the Urine of Rabbit—To the crude glucuronide gum which was obtained from 24 hr. urine of 10 rabbits administered totally 10.5 g. of ethanamide (0.3 g./kg. body wt.), 5 ml. of H₂O was added and the mixture was extracted with AcOEt (100 ml x 10). After drying over anhyd. Na₂SO₄, it was evaporated to dryness in vacuo, leaving 3.6 g. crude N-glucuronide. It was submitted to countercurrent distribution between BuOH and H₂O using 7 transfers. The contents of the fourth to the sixth were combined (1.3 g.), dissolved in 40 ml. of H₂O, and filtered. The filtrate was extracted with ether (50 ml x 6) and then with AcOEt (50 ml x 8). The latter extract was dried over anhyd. Na₂SO₄, and evaporated to dryness in vacuo. It was converted to methyl acetyl derivative by usual method. To 0.3 g. of this derivative, 15 ml. of abs. MeOH and 0.34 ml. of 2N MeONa solution was added. The mixture was small allowed to stand overnight in a refrigerator and evaporated to dryness in vacuo. To this residue a volume of MeOH was added, rubbed with a glass rod, and the crystalline substance formed on standing overnight was reprecipitated from ac. EtOH to colorless needles. Yield: 39 mg. It did not show a definite m.p. up to 250° and began to decompose from about 190°. IR λmaxν (νOH, νNH): 4.73 (νC=O), 5.76 (νC-O), 6.16 (νCCO), 6.44 (δNH). Anal. Calcd. for C₁₂H₂₄O₇N₂Na·2½H₂O: C, 43.90; H, 6.10. Found: C, 44.10; H, 5.93.

After its hydrolysis with acid, glucuronic acid could be detected by the paper chromatography, but not ethanamide because of its instability in acid.

Paper Chromatography of Carbamate N-Glucuronides—The paper chromatography was performed by ascending method using the filter paper, Toyo Roshi No. 50 and the solvent system of BuOH-AcOH-H₂O (4:1:5). The substances were visualized by Ehrlich’s reagent for II and V, and Tollens reagent for VII. NaI₂-benzidine reagent was also used in each case. The Rf values were 0.57, 0.43 and 0.68 for II, V and VII, respectively. II and VII showed the same Rf values as their corresponding free forms, I and VI, respectively.

Preliminary Test of Pharmacological Action of Sodium Meprobamate-N-mono-β-D-glucopyranosiduronate (II)—According to F.M. Berger’s method,11) the anticonvulsant effect of II was examined using white female mice of the ddN strain weighing 17 to 24 g.

The intraperitoneal administration of II (2 g./kg.) did not prevent the convulsion with strychnine (2.5 mg./kg.) dosing after a 30 min. interval, while meprobamate (500 mg./kg.) was markedly effective on the control experiments.

Periodate Oxidation of Carbamate N-Glucuronides—To a solution of 0.188 g. of sodium meprobamate N-mono-β-D-glucopyranosiduronate (II) in 100 ml. of 40% dioxane, 0.214 g. of NaI₂ was added. The reaction mixture was allowed to stand at 4° in the absence of light.

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<td>Sodium meprobamate N-mono-β-D-glucopyranosiduronate</td>
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<td>Sodium urethane N-mono-β-D-glucopyranosiduronate</td>
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<tr>
<td>2-Naphthyl-β-D-glucofuranosiduronamide</td>
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<td>Moles of NaI₂ consumed</td>
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a) The data were quoted from the reference 4.

Sodium urethane N-β-n-glucopyranosiduronate (V) (0.137 g.) was treated with 0.214 g. of NaIO₄ in the same manner as that of II. At intervals samples were withdrawn and the amount of periodate consumed was determined by Fleury and Lange's method.\(^{(12)}\)

The amount of formic acid liberated was also estimated by titration with 0.01N NaOH after decomposition of the excess of periodate with ethyleneglycol. The results are shown in Table I.

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**Summary**

Amorphous meprobamate N-glucuronide, a metabolite of meprobamate which was reported in the previous paper was converted to crystalline sodium salt and the structure was reconfirmed to be sodium meprobamate N-mono-β-n-glucopyranosiduronate.

It was also proved that ethinamate (1-ethynylethylhexylcarbamate) formed the corresponding N-glucuronide in rabbits after dosing the drug. Therefore it could be concluded that N-glucuronide formation might be one of the metabolic pathways in animal body for the drug possessing a carbamate group, although it was not the case for urethane which decomposed almost completely in animal body.

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