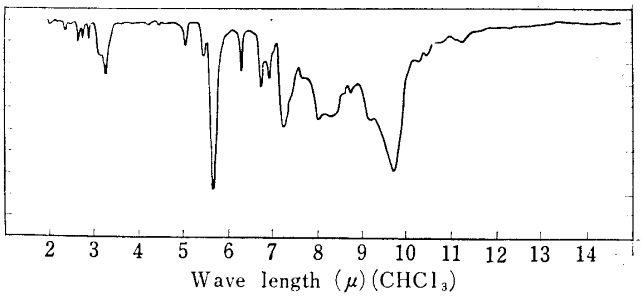
from 5 g. of nitrosomethylurea, was added to a solution of 18 mg. of purified glucuronide dissolved in 2 cc. of MeOH, the mixture was allowed to stand overnight in a refrigerator, and a small amount of precipitate formed was removed by filtration. The filtrate was evaporated to dryness in a reduced pressure to yield a pale brown material, which was dissolved in 5 g. of pyridine and 4 g. of Ac_2O was added to the solution. After the mixture was allowed to stand at room temperature for 5 days, it was poured into ice-water with stirring and extracted 3 times with Et_2O. The combined Et_2O extract was washed successively with dil. HCl and H_2O, dried over anhyd. Na_2SO_4, and evaporated to dryness. The residue was dissolved in MeOH and the solution was passed through alumina column. The column was eluted with CCl_4 and with MeOH. The MeOH solution was evaporated to dryness and the residue was dried over P_2O_5 *in vacuo* for 3 days. The solidified derivative of the glucuronide was recrystallized twice from iso-PrOH, m.p. 75~78°. Yield, 11.0 mg. *Anal.* Calcd. for $C_{22}H_{29}O_{12}N$: C, 52.90; H, 5.86; N, 2.81. Found: C, 52.13; H, 6.02; N, 2.44.

Infrared absorption spectrum of the derivative is shown in Fig. 1 and the absorptions indicate the loss of a free OH band at 3650~3590 cm^{-1} and appearances of bands characteristic to C-O in acetate at 1250 cm^{-1}, C=O in normal saturated ester at 1752 cm^{-1}, and C-O-C in a six-membered ring at 1035 cm^{-1}.



250

Fig. 1. Infrared Absorption Spectrum of Methyl [(4-Ethynyl-4-carbamyloxycyclohexyl) tri-O-acetyl-β-D-glucopyranosid]uronate

The author expresses his deep gratitude to Prof. H. Tsukamoto, Kyushu University, for his kind encouragement in this work. He is indebted to the members of the Analysis Room of the Institute of Pharmaceutical Sciences, Kyushu University, for infrared spectral measurement, and to Miss S. Fujishima in this Faculty for microanalysis.

Summary

Methyl [(4-ethynyl-4-carbamyloxycyclohexyl) tri-O-acetyl-β-D-glucopyranosid]uronate was prepared from glucuronide which had been obtained from the urine of man receiving 1-ethynylcyclohexyl carbamate.

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Toshiro Murata: Metabolic Fate of 1-Ethynylcyclohexyl Carbamate. V.[*1] Studies on 1-Ethynyl-1,2-cyclohexanediol in the Urine of Man receiving 1-Ethynylcyclohexyl Carbamate.

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In the previous paper of this series,[1] excretion of 1-ethynyl-1,2-cyclohexanediol in the urine of man who received 1-ethynylcyclohexyl carbamate (ECC) was suggested and synthesis of cyclohexanediols was attempted.

[*1] Part IV. This Bulletin, 9, 334 (1961).

[*2] Kuhonji, Ōe-machi, Kumamoto (村田敏郎).

[1] Part III. T. Murata: This Bulletin, **9**, 167 (1961).

In the present work, the metabolite was isolated and proved to be 1-ethynyl-*trans*-1,2-cyclohexanediol by comparison with the synthetic samples which had already been reported. The metabolite isolated from the urine was reactive with Horrocks reagent²⁾ as well as with the Tollens reagent, and paper chromatography of the metabolite in ascending development with two kinds of solvent system gave the same Rf values as those of 1-ethynylcyclohexanediols. The Rf value of the metabolite agreed with that of 1-ethynyl-*trans*-1,2-cyclohexanediol in the solvent system of butanol-acetic acid-water (4:1:5). As the metabolite did not crystallize, it was reduced over palladium on calcium carbonate and was purified by paper chromatography. The product thus obtained was an oily substance and did not react with the Tollens reagent, but reacted with the Horrocks reagent. This hydrogenated metabolite was compared with synthetic 1-ethylcyclohexanediols. In many respects, such as Rf values in paper chromatography with three kinds of solvent system and migration distance in paper electrophoresis, the reduced metabolite was identical with 1-ethyl-*trans*-1,2-cyclohexanediol.

Experimental

Isolation of the Metabolite (I)——About 2 L. of urine was collected from men who had received a combined total oral dose of 59 g. of ECC, 8 hr. before the collection. The extraction and alumina chromatographic procedure were the same as those used for the isolation of 1-ethynyl-4-hydroxycyclohexyl carbamate (OH-ECC).³⁾ After elution of OH-ECC with benzene-CH_2Cl_2 mixture, the alumina column was eluted with MeOH. The methanolic eluate was evaporated in a reduced pressure and 1.7 g. of a dark brown oily substance was obtained. The product was distilled in a reduced pressure and the distillate boiling out above 50°/10 mm. Hg was collected. The product (I) came as a pale yellow oily substance and reactive with both the Tollens and Horrocks reagents.

Catalytic Hydrogenation of (I)——Total amount of about 0.2 g. of (I), obtained as above, was dissolved in 10 cc. of MeOH and reduced with H_2 over 0.1 g. of pre-reduced Pd-$CaCO_3$ catalyst. The reaction mixture was filtered and MeOH was removed from the filtrate. A faint yellow oily substance was obtained, which did not react with the Tollens reagent, but reacted with the Horrocks reagent. Yield, 0.13 g.

Purification of Reduced Metabolite——The reduced product was developed on Tōyō Roshi No. 50, using the solvent system (A) of BuOH-AcOH-H_2O (4:1:5) for 18 hr. From the paper, a fraction corresponding to Rf range of 0.7~0.9 was cut off and the paper was extracted with Et_2O for 6 hr. The extract was evaporated to dryness, the residue was dissolved in hot hexane, and the white crystals that formed were removed by filtration. The filtrate was evaporated to dryness in a reduced pressure and about 70 mg. of oily substance (II) was obtained.

Identifications of (I) and (II) with Synthetic Samples——The synthetic 1-ethynyl-*trans*-1,2-cyclohexanediol (III), 1-ethynyl-*cis*-1,2-cyclohexanediol (IV), 1-ethyl-*trans*-1,2-cyclohexanediol (V), and 1 ethyl-*cis*-1,2-cyclohexanediol (VI) were prepared according to the method reported in a previous paper¹⁾ and were used as samples.

Paper chromatography: Ascending development was employed with Tōyō Roshi No. 50. Solvent systems used were (A) as described above, (B) BuOH saturated with NH_3, and (C) BuOH-EtOH-AcOH (6:2:3). The paper chromatograms were detected by spraying with $NaIO_4$ followed by benzidine

Table I. Paper Chromatography and Paper Electrophoresis of Compounds (I) to (VI)

Compd. No.	Paper chromatography (solvent system) Rf (A)	(B)	(C)	Paper electrophoresis MD (cm.)
(I)	0.90		0.89	
(III)	0.90		0.89	
(IV)	0.95		0.89	
(II)	0.82	0.87	0.86	−3.6
(V)	0.81	0.87	0.86	−3.7
(VI)	0.86	0.91	0.92	−2.9

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2) R. H. Horrocks: Nature, **164**, 444 (1949).

3) T. Murata: This Bulletin, 8, 629 (1960).

reagents prepared according to Horrocks.[3] The results are shown in Table I.

Paper electrophoresis : The horizontal open-strip type apparatus (plastic frame support) was used with constant voltage. The current of 500 V was applied to the filter paper strip (12×38 cm.) and charged for 4 hr. Electrolyte system used was 1% borax solution. The results are listed in Table I.

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Summary

A metabolite of 1-ethynylcyclohexyl carbamate other than 1-ethynyl-4-hydroxycyclohexyl carbamate and its glucuronide was isolated from urine of man receiving the drug. The metabolite was identified as 1-ethynyl-*trans*-1,2-cyclohexanediol by comparing the metabolite and its reduced product with synthetic samples. Paper chromatography with three kinds of solvent system and paper electrophoresis were employed for the identification.

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