Notes

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Toshiro Murata, Shōko Etō, and Setsuko Fuchigami : Metabolic Fate of 1-Ethynylcyclohexyl Carbamate. VI.*1 Production of 1-Ethynylcyclohexane-*trans*-1,2-diol from 1-Ethynylcyclohexyl Carbamate *in vitro*.

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In the previous papers of this series,[1] Murata has reported the urinary excretion of 1-ethynylcyclohexane-*trans*-1,2-diol (Ⅳ) other than 1-ethynyl-4-hydroxycyclohexyl carbamate (Ⅱ) and its O-glucuronide (Ⅲ) from human who received 1-ethynylcyclohexyl carbamate (Ⅰ) orally.

The production of Ⅳ from Ⅰ was further confirmed by perfusing Ⅰ and 1-ethynylcyclohexanol (Ⅴ) through rabbit liver by the authors.

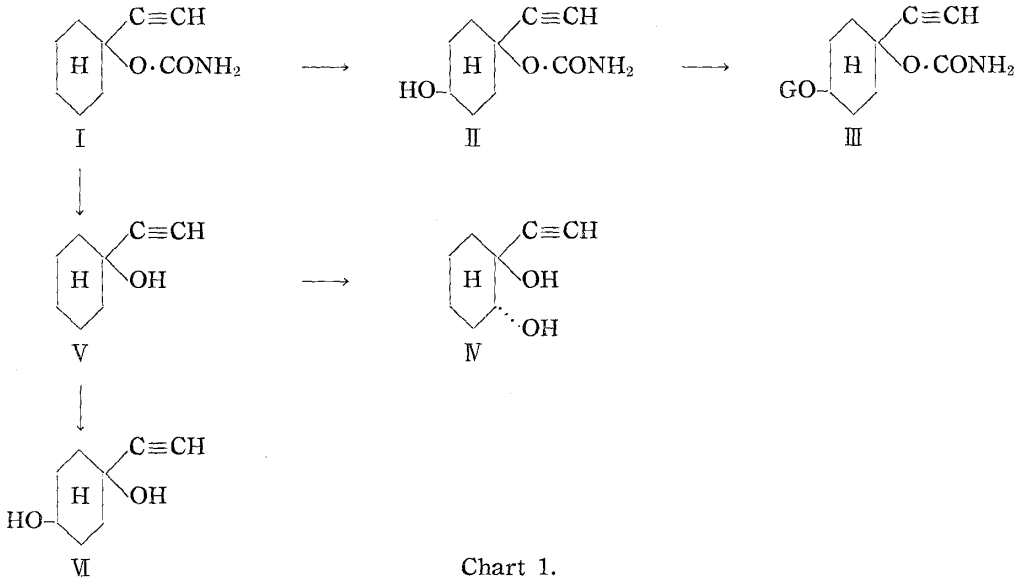

Chart 1.

The metabolites obtained by perfusion were identified by a gas chromatography.

Experimental

Materials——Ⅰ was a commercial sample and recrystallized before use. Ⅳ, Ⅴ and 1-ethynylcyclohexane-1,4-diol (Ⅵ) were synthesized by the methods as described before.[1] Ⅱ was a metabolite of Ⅰ and it was obtained from human who received Ⅰ as described in the previous paper.[2]

Rabbit Liver Perfusion——Twenty rabbits were used in this study. Rabbits weighing 2~3 kg. were sacrificed by bleeding after fasting for 20 hr., and immediately after death the liver was removed. The liver perfusion was carried out with the rabbit defibrinated blood diluted with Locke's solution.[3]

Two hundreds mg. of each sample were perfused in a experiment. The perfused blood solution was acidified with HCl to pH 1.0 and hydrolyzed by warming in a boiling bath for 1.5 hr. The hydrolyzed

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*1 Part Ⅴ : This Bulletin, **9**, 335 (1961).

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1) T. Murata : This Bulletin, **9**, 167 (1961).

2) *Idem* : *Ibid.*, 8, 629 (1960).

3) S. Tsuboi : Seikagaku, **21**, 34 (1949).

solution was extracted with Et_2O, and the extract was evaporated to dryness under reduced pressure. The residue thus obtained was dissolved in a small amount of Me_2CO and was filtrated. The filtrate was used as a sample for gas chromatography as follows.

Gas Chromatography——A Shimadzu Seisakusho Model GC-1B, HFD-1 instrument (dual column, differential flame) was used in this study. A stainless steel column of 225 cm. × 4 mm. i. d. was packed with 1.5% SE-30 on Chromosorb W (80~100 mesh). All chromatographies were carried out under the conditions as follows : column temp. 135°, sample heater temp. 250°, detector temp. 150°, carrier gas N_2, 12.5 ml./min.

Results and Discussion

The chromatogram of the metabolites of V obtained by perfusing rabbit liver is shown in Fig. 1, and standard chromatogram of authentic samples in acetone is shown in Fig. 2. respectively. In Fig. 1. it is found that the chief metabolite of V is Ⅳ, however, *cis*- and *trans*-isomer of the diol is not distinguished on the chromatogram. A small amount of *p*-diol is also found as a metabolite of V.

Thus, it was clarified in this study that Ⅳ was a chief metabolite of V, while Ⅱ was that of I as reported before,[2] so that the Ⅳ which was obtained in previous experiment*[1] was supposed to be produced through V which was an intermediate and obtained by hydrolysis of carbamyl ester linkage in I *in vivo*.

Furthermore, the results suggested that a free alcohol (V) was more easily hydroxylated at *ortho*-position of cyclohexane ring as in the cases of other cyclohexanols,[4] than at *para*-position.

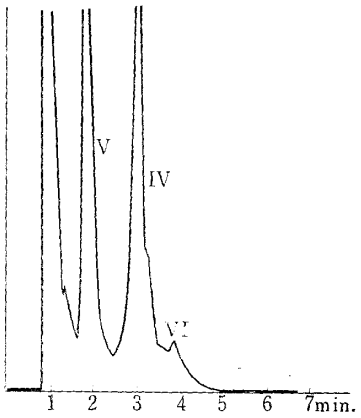

Fig. 1. Gas Chromatogram of V and its Metabolites obtained through Rabbit Liver

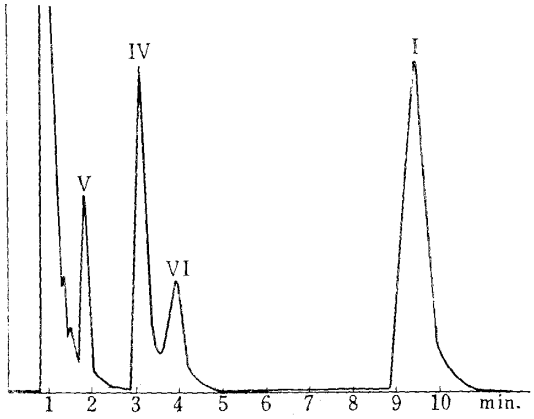

Fig. 2. Gas Chromatogram of I and its known Metabolites

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Summary

1-Ethynylcyclohexanol was perfused through rabbit liver, and the metabolites of it thus obtained were separated and identified by gas chromatography. A chief metabolite of the 1-ethynylcyclohexanol was ascertained to be 1-ethynylcyclohexane-1,2-diol, and this finding was well coincident with the result obtained from previous experiment. Furthermore, the production of 1-ethynylcyclohexane-1,4-diol from 1-ethynylcyclohexanol was also confirmed in this study, and the metabolic pathways of those were discussed.

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4) T. H. Elliott, D. V. Parke, R. T. Williams : Biochem. J., **72**, 193 (1959).