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108. Toshiro Murata : Metabolic Fate of 1-Ethynylcyclohexyl Carbamate. I. Metabolic Products in the Urine of Humans and Rabbit receiving 1-Ethynylcyclohexyl Carbamate.

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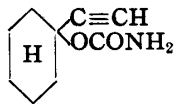
Langecker, Schumann, and Junkmann[1] examined a number of carbamates of tertiary, unsaturated carbinols and found hypnotic action in ethynylcyclohexyl carbamate (I). Its clinical use as a rapid- and short-acting, and non-toxic hypnotic was described by Tewes,[2] Schulz and Schirren,[3] and Franke.[4]



(I)

This hypnotic has found popular use in Japan and is becoming a problem in the fields of forensic chemistry and pharmacology. For this reason, a series of examinations was made on the metabolism of ethynylcyclohexyl carbamate.

Swanson, *et al.*[5] examined metabolism of ethynylcyclohexyl carbamate (I) and suggested that it would have a rapid onset of action and its duration of action would be considerably short. In their attempt to confirm this suggestion, the urine from dogs receiving oral doses of as much as 200 mg./kg. of (I) was examined by the method of Langecker, *et al.*[1] and of Perlman and Johnson,[6] but they failed to obtain the characteristic silver precipitate and only a small amount of oily black precipitate was produced. As a result, they concluded that neither (I) nor any acetylenic degradation product was excreted in the urine of a dog.

Metabolic change of (I) was followed chemically by McMahon[7] who used (I) labeled with ^{14}C in the carbonyl of $-CONH_2$ and he isolated a metabolite with unchanged carbamyl group from the urine of man and a rat. He assumed that this metabolite is probably a monohydroxyl derivative of (I).

In his studies of related ethynyl compounds with the Tollens reagent, hydroxylated (I) did not actually give any precipitate but a brown solution, while ethynylcyclohexanol and (I) respectively produced white and brown, heavy precipitates. Consequently, he suggested that the inability of previous workers to detect ethynyl compounds in the urine was due to the use of methods which all depended on the formation of an insoluble silver complex that was not formed in the case of hydroxylated (I).

In the present series of work, examinations were made on the metabolites of (I) in the urine by paper chromatography, using the Tollens reagent. A metabolite was isolated from the urine of man receiving (I) orally and the metabolite was identified as hydroxylated (I) which had been obtained by McMahon.

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1) H. Langecker, H. J. Schumann, K. Junkmann : Arch. exptl. Pathol. Pharmakol., **219**, 130(1953).
2) H. Tewes : Arztl. Praxis, **6**, 1(1954).
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4) G. Franke : Med. Klinik. (Munich), **49**, 891(1954).
5) E. E. Swanson, R. C. Anderson, W. R. Gibson : J. Am. Pharm. Assoc., Sci. Ed., **45**, 40(1956).
6) P. L. Perlman, C. Johnson : *Ibid.*, **41**, 13(1952).
7) R. E. McMahon : J. Am. Chem. Soc., **80**, 411(1958).

Materials and Methods

Urine of Rabbits—Rabbits weighing 2~3 kg. were administered with 0.5 g. of (I) in the form of a suspension in 0.2% sodium alginate solution by means of a stomach tube. Total urine excreted during 24 hr. after administration was collected and used as a sample.

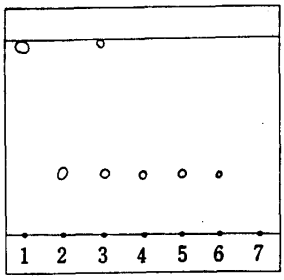
Urine of Men—Daily dose of 0.5~1.5 g. of (I) was given orally to healthy persons before retiring and the first urine excreted the following morning (after about 8 hr.) was collected. The urine sample was used for both paper chromatography and isolation of metabolites.

Paper Chromatography of the Metabolites—Paper chromatography was carried out by the ascending method, using Toyo Roshi No. 50 filter paper, and developed with basic and acid solvents, the basic solvent being BuOH saturated with 28% NH_4OH and the acid solvent, a mixture of BuOH-EtOH-0.5*N* AcOH (6:2:3). Substances on the paper chromatogram were detected by spraying the Tollens reagent prepared by mixing 125 cc. of 0.1*N* $AgNO_3$, 15 cc. of 6*N* NaOH, 20 cc. of 28% NH_4OH, and 90 cc. of H_2O, or by contacting with I_2 vapor.

Hydrolysis of Human Urine—Urine sample from men receiving (I) was boiled for 1 hr. with either 1 g. of NaOH or 0.6 cc. of conc. H_2SO_4 for 50 cc. each of sample urine.

Experimental and Results

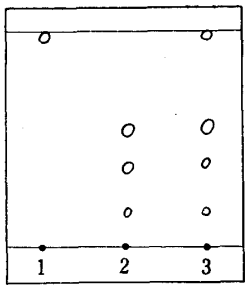
Paper Chromatography of Rabbit Urine—The paper was developed for a short period with either basic or acid solvent system. When the basic solvent was used, only one metabolite was detected on the paper as a brown spot with the Tollens reagent, independent of the pH of sample urine, as shown in Fig. 1. When developed with the acid solvent, the Tollens reagent gave three spots on chromatogram (Fig. 2).



1. EtOH solution of (I)
2. Urine collected during 24 hr. after administration of (I)
3. Sample 2 added with (I)
4. Sample 2 acidified with HCl to pH 3.0
5. Sample 2 made alkaline with NaOH to pH 10.0
6. Urine collected after 24 hr.
7. Rabbit urine not administered with (I)

BuOH saturated with NH_4OH

Fig. 1. Paper Chromatogram of Rabbit Urine



1. EtOH solution of (I).
2. Urine collected during 24 hr. after administration of (I)
3. Sample 2 added with (I)

BuOH-EtOH-0.5*N* AcOH (6:2:3)

Fig. 2. Paper Chromatogram of Rabbit Urine

Paper Chromatography of Human Urine—The urine sample (pH 8.6) was developed for 24 hr. with both the solvent systems. When using the basic solvent, spots were obtained with the Tollens reagent and the same number of clear yellow spots were obtained by contacting with I_2 vapor in almost the same positions as those detected by the Tollens reagent. When developed with the acid solvent, however, separation of the spots was unsatisfactory as shown in Fig. 3. Therefore, the basic solvent was used for subsequent paper chromatography.

Among the six spots obtained with the Tollens reagent, three were found to be not the metabolites of (I) by comparison with the paper chromatogram of the human urine not receiving (I). The

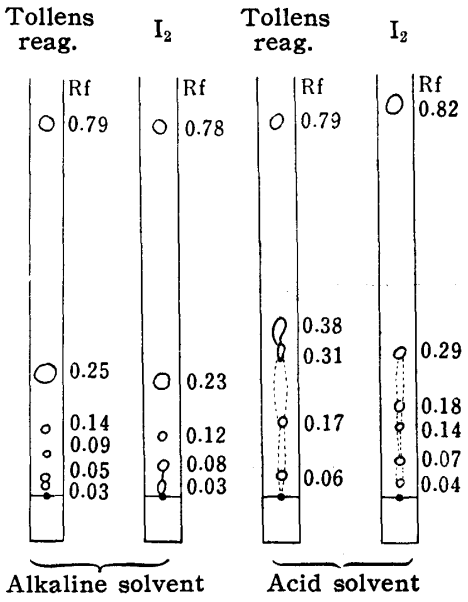


Fig. 3. Paper Chromatograms of Urine from Men Receiving Ethynylcyclohexyl Carbamate

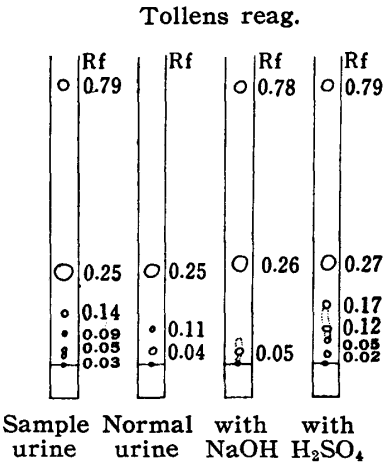


Fig. 4. Paper Chromatograms of Urine from Men Receiving Ethynylcyclohexyl Carbamate after Hydrolysis

one of remaining three spots disappeared on boiling the sample urine with NaOH but the chromatogram was not affected when the urine was treated with H_2SO_4. These results are shown in Fig. 4.

Isolation of the Metabolites of (I)—Extraction of metabolites of (I) from human urine was attempted by the route shown in Chart 1. The urine sample was first basified to pH 11.0 with NH_4OH and extracted with Et_2O or CH_2Cl_2. The extraction residue was acidified to pH 3.0 with HCl and again extracted with Et_2O or CH_2Cl_2. The combined extract was evaporated to dryness, the brown

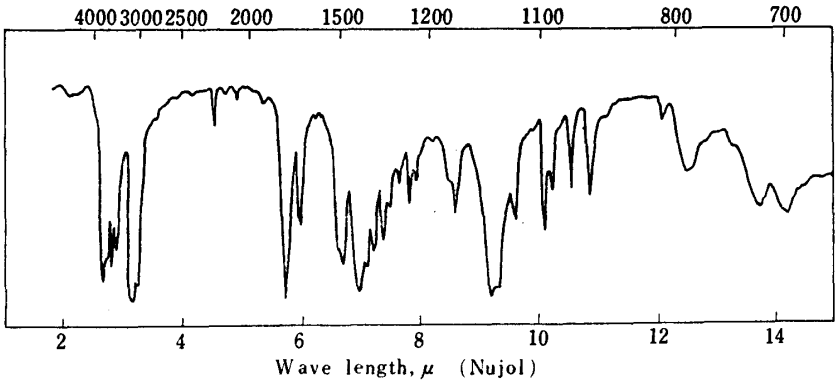


Fig. 5. Infrared Absorption Spectrum of Hydroxylated (I)

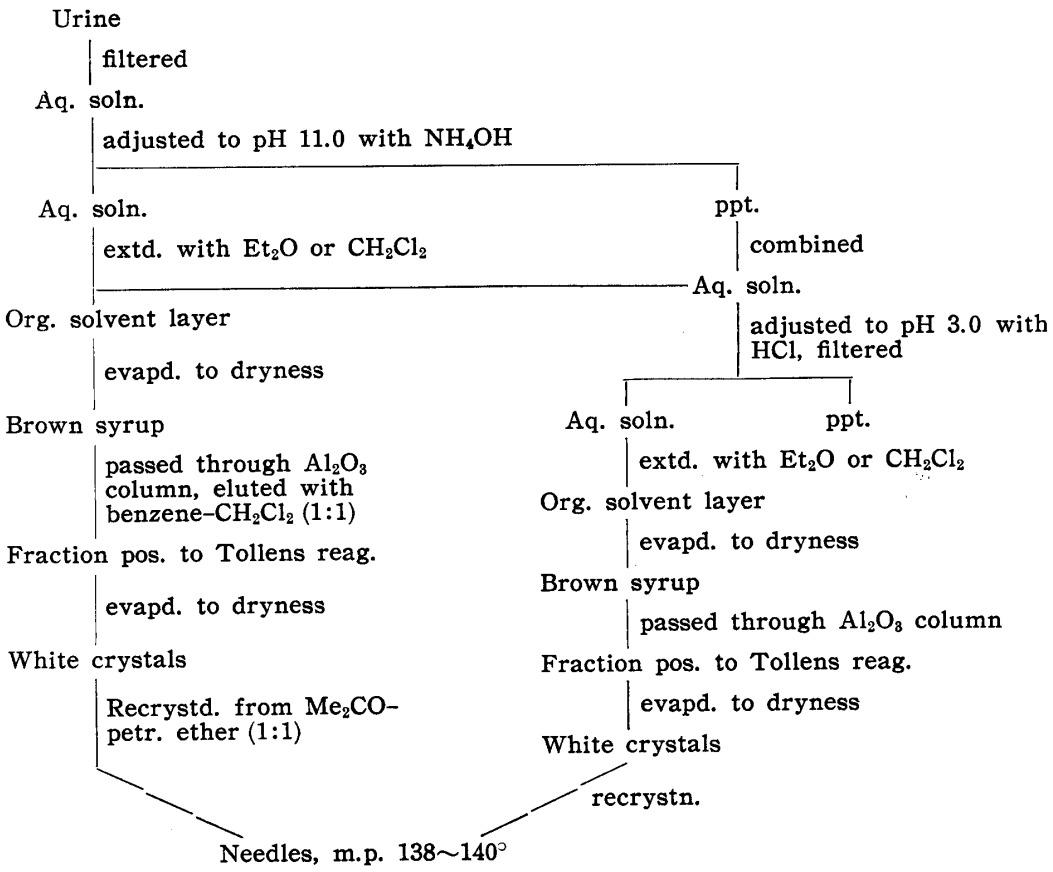


Chart 1.

oily residue was dissolved in a small amount of CH_2Cl_2, and the solution was passed through a column (1.2×40 cm.) of alumina. The column was eluted with benzene-CH_2Cl_2 (1:1) mixture and effluents giving positive reaction to the Tollens reagent were collected. The solvent was removed from the effluent and the white crystalline residue was recrystallized from Me_2CO-petr. ether (1:1) to colorless needles, m.p. 138~140°. *Anal.* Calcd. for $C_9H_{13}O_3N$: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.94; H, 7.28; N, 7.36.

This metabolite was found to have an ethynyl group as characterized by the Tollens reagent and its m.p. was very close to that of monohydroxylated (I), m.p. 135~136°, obtained by McMahon.[7]

Preparation of Derivatives of the Metabolic Product—Two kinds of derivatives of the crystalline metabolite were prepared according to the method reported by McMahon.[7]

i) Catalytic hydrogenation of the metabolite: A solution of 200 mg. of the metabolite dissolved in 20 cc. of EtOH, added with 0.1 g. of pre-reduced Pd-$CaCO_3$, was shaken in H_2 stream. Uptake of H_2 ceased after absorption of 60.8 cc. in 1.5 hr. The catalyst was filtered off, the filtrate was evaporated to dryness, and two recrystallization of the residue from benzene gave 46 mg. of the product as crystals of m.p. 133~134°. *Anal.* Calcd. for $C_9H_{17}O_3N$: N, 7.48. Found: N, 7.33.

ii) Acetylation of the metabolite: A solution of 50 mg. of the metabolite dissolved in 5 cc. of dehyd. pyridine and 1 cc. of Ac_2O was allowed to stand at room temperature for 3 days. Et_2O was added to this solution, Et_2O layer was washed with water, and evaporated to dryness. Recrystallization from benzene gave colorless crystals, m.p. 153~155°. Yield, 22 mg. *Anal.* Calcd. for $C_{11}H_{15}O_4N$: C, 58.65; H, 6.71; N, 6.22. Found: C, 58.20; H, 6.93; N, 6.01.

Paper Chromatography of the Metabolite—A solution of the metabolite dissolved in EtOH was chromatographed and spots at Rf 0.77~0.81, shown in Figs. 3 and 4, were identified as those of the metabolite extracted from the human urine after administration of (I). Presence of acetylated metabolite (Rf 0.87) was not recognized in the human urine after administration of (I).

Discussion

A spot revealed by the Tollens reagent on the paper chromatogram of the human urine after oral administration of (I) was identified as hydroxylated (I) which was isolated

from human urine and confirmed by elementary analyses, melting point, infrared spectrum (Fig. 5), and preparation of two derivatives.

One of the other spots (Rf 0.03 in Fig. 4) disappeared on hydrolysis of the urine with 2% sodium hydroxide. McMahon had observed disappearance of the spot with a low Rf value on radioautograms on treating the urine sample with β-glucuronidase and supposed that the metabolite is a glucuronide of hydroxylated (I). If the urinary metabolites of (I) contained glucuronide of hydroxylated (I), the glucuronide should be an ether type and extraction of hydroxylated (I) from the urine in basic condition, as reported by McMahon would be unsatisfactory. This point was confirmed by the present series of experiments, result of which is shown in Table I. The metabolite, hydroxylated (I), was recovered by extraction after acidification of the alkaline extraction residue of the urine. Cleavage of the conjugated form of hydroxylated (I) appeared to be insufficient in the first extraction and the metabolite must have been further hydrolyzed by subsequent acidic extraction.

Table I. Extraction of Metabolites from Human Urine

Combined total oral dose (g.)	Combined Total amount of urine (cc.)	pH of urine	Solvent	Yield of metabolite (g.)
45	17,162	11.0	Et_2O	0.28
54	19,875	3.0	〃	0.12
59	19,675	11.0	CH_2Cl_2	0.57
59	19,675	3.0	〃	0.07

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Summary

Paper chromatography was carried out on the urine of rabbits and men receiving 1-ethynylcyclohexyl carbamate. The chromatogram revealed the presence of six spots by the Tollens reagent and three of them were assumed to be the metabolites, degradation products of the carbamate. One of them was identified as the hydroxylated ethynylcyclohexyl carbamate which was also obtained by extraction of the urine of man receiving 1-ethynylcyclohexyl carbamate. Another spot on the chromatogram was assumed to be that of a conjugated form of the metabolite such as the glucuronide which had been reported by McMahon.

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