

Metabolic Fate of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone. II.^{1,2}
Metabolism of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone
by Rabbits Liver

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Two metabolites were isolated as metabolic products of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (MTQ) in the 9000×*g* supernatant fraction of rabbits liver. These products were identified as 2-methyl-3-*o*-hydroxymethylphenyl-4(3*H*)-quinazolinone (MHQ) and 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone N-oxide (MTQNO) by comparing with authentic samples. Ultraviolet absorption spectra, fluorescence spectra, paper chromatography and thin-layer chromatography with seven kinds of solvent system and paper electrophoresis were employed for the identification.

In the preceding paper,¹⁾ the authors have reported that a metabolite of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (MTQ) was isolated from the urine of man receiving the drug, and its chemical structure was confirmed as 2-nitrobenzo-*o*-toluidide (NBT).

The studies presented in this paper was carried out in order to clarify the biotransformation process of NBT and other metabolic pathway *in vitro*. The result of this experiment shows that MTQ is metabolized with the supernatant fraction (9000×*g*) contained microsomes and soluble fraction, in the presences of NADPH₂ and O₂. 2-Methyl-3-*o*-hydroxymethylphenyl-4(3*H*)-quinazolinone (MHQ), and 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone N-oxide (MTQNO) were detected in the reaction mixture.

Materials and Methods

Materials

MTQ was supplied from Eisai Co., Ltd. (Tokyo), mp 113—115°. MHQ, mp 109—110°, was supplied through the courtesy of Prof. Akagi and Dr. Oketani (Faculty of Pharmaceutical Sciences, University of Hokkaido). MTQNO was synthesized according to the method of Toyoshima, *et al.*⁴⁾ NADPH₂ was purchased from Sigma Chemical Company. Alumina (Brochmann) was purchased from E. Merck Ag. Darmstadt.

Methods

Preparation of Tissue Sample—The preparation of tissue samples was carried out at 0—3°. Male albino rabbit was sacrificed by bleeding after fasting for 24 hr. Liver was removed and homogenized for 1 minute in a teflon pestle glass homogenizer with 2 volumes 1.15% (isotonic) KCl solution. The 9000×*g* supernatant was prepared by differential centrifugation of the homogenate.

Incubation of MTQ with Tissue Sample—An incubation mixture was consisted of 3.0 ml of the supernatant fraction (equivalent to 1 g of liver), 10 μmole of MTQ, 100 μmole of nicotinamide, 5 μmole of NADPH₂, 100 μmole of MgCl₂, 7 ml of 0.2M phosphate buffer (pH 7.4) and water to final volume of 13 ml. The reaction mixture were deprotenized with 40% trichloroacetic acid, centrifuged, basified with 28% NH₄OH and extracted with CHCl₃ successively. The CHCl₃ layer was evaporated to dryness under reduced pressure.

Synthesis of MTQNO—To a solution of 12.5 g (0.1M) of MTQ dissolved in 140 ml of AcOH, 10 ml of 30% hydrogen peroxide were added and was stirred at 55—60° for 5 hr. After 5 hr, 8 ml of 30% hydrogen

1) Part I: T. Murata and I. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 133 (1970).

2) This was reported at the 86th Annual Meeting of Pharmaceutical Society of Japan, Toyama, April 1966.

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4) S. Toyoshima, S. Hamano and K. Shimada, *Yakugaku Zasshi*, **85**, 507 (1965).

peroxide were added again and the reaction was continued for 50 hr. After the reaction was over, the solvent was distilled off under reduced pressure. The dark red residue thus obtained was dissolved in benzene, and was applied on a column of alumina treated with 10% AcOH. The column was eluted with benzene, and benzene-acetone (9:1), successively. Each effluent was submitted to the thin-layer chromatography with CHCl_3 -acetone (9:1), and the fractions which contained MTQNO (R_f 0.22) were collected. The solvent was evaporated under reduced pressure, and the residue was recrystallized from acetone to yellow crystallines, mp 187–189° (decomp.). Yield, 2.60 g. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_2$: C, 72.24; H, 5.31; N, 10.53. Found: C, 71.77; H, 5.54; N, 10.12.

Paper and Thin-Layer Chromatography—Ascending paper chromatography was carried out for the identification of metabolic products using the following solvent systems: (A) BuOH saturated with 28% NH_4OH and (B) BuOH-AcOH- H_2O (4:1:5). The chromatogram was examined under ultraviolet light irradiation. Some spots of the metabolites was detected as dark spots or yellow spots.

By spraying Dragendorff reagent on the paper chromatogram, MTQ and its metabolite having quinazolinone ring showed orange yellow spots on white or pale yellow background. I_2 vapor was also used for the detection of the chromatogram.

Thin-layer chromatography was carried out using the materials and solvent systems as follows: Mercks silica gel G was applied on a glass plate, 20 × 20 cm, with the applicator of Mitamura Co., Ltd. to make a thin-layer of 0.25 mm and dried at 105° for 3 hr. The chromatograms were developed by using the solvent systems; (C) hexane-methylene chloride-EtOH (6:3:1) (D) CHCl_3 -acetone (9:1) (E) benzene-ethyl acetate (9:1) (F) CHCl_3 -pyridine (3:1) (G) ethyl acetate-EtOH (9:1). For detecting the chromatograms, the same method as in paper chromatography was used.

Paper Electrophoresis—The samples were spotted on the center of Toyo Roshi No. 50 filter paper (10 × 39 cm) and the electrophoresis conducted between sheet of glass using 1% NH_4OH as the medium, running time 3.5 hr, 10 mA (1 mA/cm) current.

Ultraviolet Absorption Spectra and Fluorescence Spectra—Ultraviolet absorption spectra were recorded by using Hitachi recording spectrophotometer Model EPS-2U, and the fluorescence spectra were measured by using Aminco-Bowman spectrophotofluorometer.

Results

Identification of the Metabolites of MTQ

The extract from the incubation mixture of MTQ with liver tissue was dissolved in a small amount of CHCl_3 and submitted to the thin-layer chromatography. Authentic samples

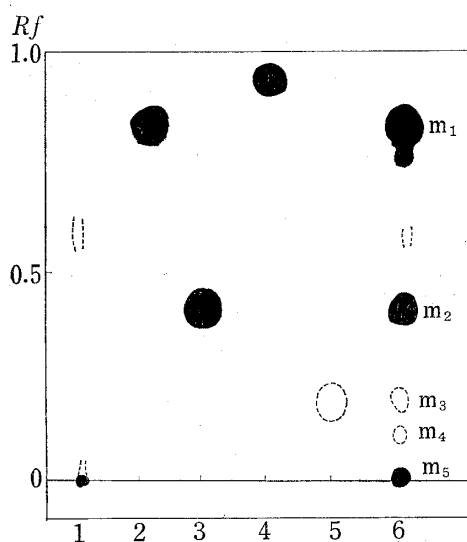


Fig. 1. Thin-layer Chromatogram of the Metabolic Products isolated from the Incubation Mixture. Solvent System (D)

1: Control 2: MTQ 3: MHQ 4: NBT
5: MTQNO 6: Metabolic Products
● : Dragendorff reagent
○ : fluorescence



Fig. 2. Ultraviolet Absorption Spectra of MTQ and the Metabolic Products

●—●: MTQ ▲—▲: m₁ ○—○: m₂
□—□: m₃ △—△: m₄ ▽—▽: m₅

of MTQ, MHQ and NBT were chromatographed simultaneously. On the thin-layer chromatogram, four spots of metabolites were found other than the spot of unchanged MTQ. The uppermost spot corresponded to the spot of MTQ (R_f 0.86) (m_1) as shown in Fig. 1.

When ultraviolet light irradiation was used for detecting method, two kinds of metabolites were detected as deep yellow spots (m_3, m_4). Other metabolites were found as orange yellow spots (m_2, m_5) with Dragendorff reagent. On contacting with I_2 vapor, all the four spots gave brown colors.

From a series of unsprayed thin-layer chromatogram plates, the corresponding all metabolites on the chromatogram were extracted with EtOH, and their ultraviolet absorption spectra were recorded as shown in Fig. 2.

Isolations and Identifications of Metabolites from the Incubation Mixture

The $CHCl_3$ extracts of the incubation mixture were combined, washed with a small volume of H_2O , dried over anhyd. Na_2SO_4 , and evaporated under reduced pressure. The residue was dissolved in a small volume of benzene, and column chromatographed through of 15 g of alumina treated with 10% AcOH, and the effluents were submitted to thin-layer chromatography, as shown in Fig. 3.

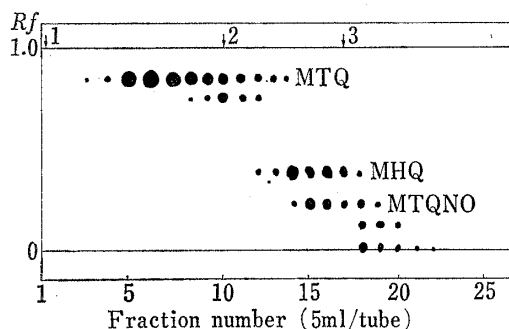


Fig. 3. Thin-Layer Chromatogram of Effluents from Alumina Column Chromatography. Solvent System (D)

solvent
1: benzene 2: benzene-acetone (9:1)
3: benzene-acetone (3:1)

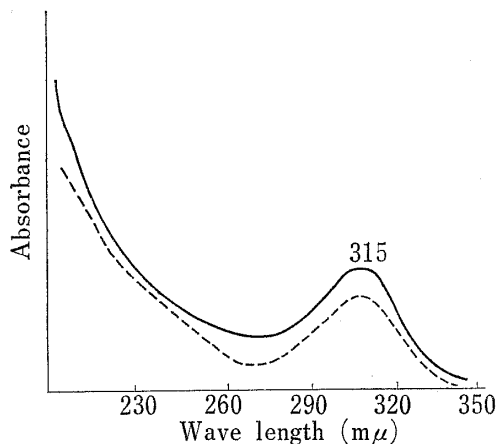


Fig. 4. Ultraviolet Absorption Spectra of Authentic MTQNO (Solid Line) and the Metabolic Product isolated from Incubation Mixture (Broken Line) in EtOH

The compound (m_3) was found to have a maximal absorption at 315 $m\mu$ and a yellow fluorescence for ultraviolet light irradiation, and was negative both for Ehrlich and Dragendorff reagent. However, it gave a brown color by contacting with I_2 vapor. And m_3 was suggested to be coincident with MTQNO by paper, thin-layer chromatography, and electrophoresis as shown in Table I.

TABLE I. Paper and Thin-Layer Chromatograms, and Paper Electrophoresis of the Metabolic Products

Solvent system	R_f values							Paper electrophoresis M.D. (cm)
	A	B	C	D	E	F	G	
Authentic MHQ	0.76	0.90	—	0.36	0.18	—	0.87	—
Metabolic Product	0.76	0.89	—	0.36	0.18	—	0.86	—
Authentic MTQNO	—	—	0.67	0.22	0.09	0.50	0.57	-1.3
Metabolic Product	—	—	0.64	0.21	0.08	0.45	0.57	-1.4

For further identification of the metabolite, the spot on the thin-layer chromatogram was scraped off and extracted with abs. EtOH. The EtOH extract was subjected to the measurements of ultra-violet and fluorescence spectra, as shown in Fig. 4 and Fig. 5.

However, NBT could not be detected under this experimental conditions. A Dragendorff positive compound with R_f 0.36 (m_2) was identified as MHQ by comparison with authentic sample as shown in Table I. This compounds had been isolated and reported as a major metabolite of MTQ from the urine of rabbits by Akagi, *et al.*⁵⁾

Discussion

The oxidation products of MTQ with rabbit liver $9000\times g$ supernatant were isolated and identified by the authors.

MTQNO and MHQ were isolated from the incubation mixture of MTQ and rabbit liver $9000\times g$ supernatant. However, NBT which was isolated from the urine of man receiving MTQ⁶⁾ by the authors was not detected in this incubation mixture.

Kiese, *et al.*,⁷⁾ Ueleke,⁸⁾ Akagi, *et al.*,⁹⁾ and Troll, *et al.*¹⁰⁾ had showed that some of amino compounds were oxidized to corresponding hydroxyamino and nitroso compounds both *in vivo* and *in vitro*.

On the other hand, the formations of N-oxide had been reported as results of biological oxidation of trimethylamine,¹¹⁾ chlorpromazine,¹²⁾ imipramine,¹³⁾ nicotinamide,^{14,15)} diallyl-melamine,¹⁶⁾ and nicotin.¹⁷⁾

Kawai, *et al.*¹⁸⁾ have found the oxidation of *p*-aminobenzoic acid to *p*-nitrobenzoic acid by *S. thioluteus*. And they have demonstrated the formation of N-oxide from *p*-(N-dimethyl)-aminobenzaldehyde by the same organism. Those findings led them to proposed the following scheme for the mechanism of nitro formation.

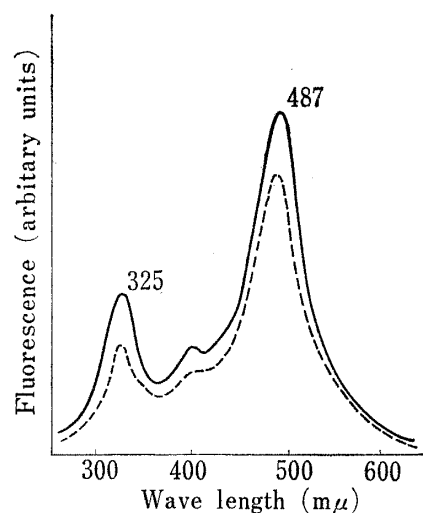


Fig. 5. Fluorescence Emission Spectra of Authentic MTQNO (Solid Line) and the Metabolic Product isolated from Incubation Mixture (Broken Line) in EtOH

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And the formation of N-oxide is often observed as an intermediary reaction of N-dealkylation *in vitro*.

Those findings described above suggest that MTQNO is a possible intermediate from MTQ to NBT *in vivo*.

The metabolic pathway is unable to confirm in this experiment *in vitro*, but the formation of N-oxide of MTQ is shown by the biological oxidation.