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Metabolic Fate of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone. I.[1,2] 2-Nitrobenzo-*o*-toluidide as an Urinary Metabolite of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone in Human

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2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (MTQ) was orally administered to human, and its urinary metabolites were examined. Two kinds of metabolite were isolated from the urine, and one of them was assumed to be 2-nitrobenzo-*o*-toluidide.

On the other hand, the same compound as this metabolite was obtained by chemical oxidation of MTQ with hydrogen peroxide in glacial acetic acid. And the chemical structure of this oxidation product of MTQ was established as 2-nitrobenzo-*o*-toluidide.

Therefore, one of the metabolites of MTQ, which were isolated from human urine by the authors, was confirmed to be 2-nitrobenzo-*o*-toluidide.

Gujral and co-workers[4–6] reported that some kinds of derivative of quinazolinone exhibited a potent hypnotic action in experimental animals. One of the most active derivative was 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone(MTQ) (I). The effect of the drug on the central nervous system was also confirmed by Boissier, *et al.*[7] and its clinical use as rapid-acting hypnotic was described by Ravina[8] and Arvers.[9]

The metabolic fate of the hypnotic drug in animals and human have been studied by some workers.[10–18]

Cohen, *et al.* reported that 2-^{14}C-MTQ was excreted in unchanged form with its degraded products,[11] and urinary radioactivities after administration of 2-^{14}C-MTQ were attributed to the unchanged form (25%), the conjugated derivatives (70%), and other metabolites (5%) in mice.[16]

Akagi, *et al.* suggested that about 20% of administered MTQ was excreted as glucronides in rabbits, but they could not find any glucronide formation in human.[13] And they have isolated a metabolite from the urine of rabbits, and confirmed the metabolite as 2-methyl-3-*o*-hydroxymethylphenyl-4(3*H*)-quinazolinone.[14]

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1) The preliminary report of this work appeared in *Chem. Pharm. Bull.* (Tokyo), **12**, 631 (1964).
2) This was reported at the 34th Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Kumamoto, September 1963.
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4) M.L. Gujral, P.N. Saxena and R.S. Tiwari, *Indian J. Med. Res.*, **43**, 637 (1955).
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Preuss, *et al.*[17] isolated 12 kinds of metabolite of MTQ from the urine of human, and four of them were confirmed as 2-methyl-3-*o*-hydroxymethylphenyl-4(3*H*)-quinazolinone, 2-methyl-3-*o*-tolyl-6-hydroxy-4(3*H*)-quinazolinone, 2-methyl-3-*o*-(4-hydroxy-2-methylphenyl)-4(3*H*)-quinazolinone, and 2-methyl-3-*o*-(3-hydroxy-2-methylphenyl)-4(3*H*)-quinazolinone, respectively.

In addition to the metabolites described above, Nowak, *et al.*[18] found 2-hydroxymethyl-3-*o*-tolyl-4(3*H*)-quinazolinone and 2-methyl-3-*o*-tolyl-8-hydroxy-4(3*H*)-quinazolinone in the urine of dogs, rats, rhesus monkeys, and rabbits.

On the other hand, Cohen, *et al.*[11,16] and Nowak, *et al.*[18] suggested that the degradation products of the quinazolinone ring, such as anthranilic acid, N-acetylanthranilic acid and *o*-toluidine, might be present in the urine as metabolites of the drug, but they have been unable to find any of them.

In this paper, a new metabolite of MTQ was isolated from human urine, and its chemical structure was confirmed as 2-nitrobenzo-*o*-toluidide(NBT) (IIa) by comparing with chemical oxidation product of MTQ by the authors.

Experimental

Administration of the Drug and Collection of Urine——Urine was supplied by healthy five persons who were given orally 200—400 mg of the drug before retiring. The control urine was provided by the same person without administration of any drug.

The first urine specimen excreted on the following morning, about 8 hr after administrating the drug, was collected.

Extraction of Metabolites——The urine sample was first adjusted to pH 3.0 with HCl and extracted with CH_2Cl_2. The extraction residue was basified to pH 11.0 with NH_4OH and extracted again with CH_2Cl_2. Each extract was evaporated to dryness under reduced pressure.

Total doses of drug administered was 57.1 g, and 55, 240 ml of sample urine was submitted to extraction.

Paper Chromatography——The extracts were dissolved in a small amount of CH_2Cl_2 and were spotted on Toyo Roshi No. 50 or No. 51 filter paper. Ascending paper chromatography was performed with the solvent system of BuOH saturated with 28% NH_4OH.

The following reagents and methods were used for detecting the paper chromatogram; (1) Dragendorff (Dr.) (2) I_2 vapor(I) (3) Ehrlich(Eh) (4) Fluorescence under the ultraviolet-light irradiation(Fl.)

Chemical Oxidation of MTQ(I) with Hydrogen Peroxide——Three ml of 30% H_2O_2 and 14 ml of AcOH were added to 2.5 g (0.01 mole) of (I), and the reaction mixture was warmed at 60—70° for 7 hr. The color of the mixture was changed from yellow to reddish brown. The excess AcOH was evaporated under reduced pressure. The residue was poured into 15% K_2CO_3 solution and extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated after drying over anhyd. Na_2SO_4. The residue was dissolved in a small amount of $CHCl_3$ and was submitted to almina column chromatography. About 0.8 g yellow needles, mp 171—173°, was obtained from a part of ether effluent and recrystallization from EtOH gave pale yellow needles (IIb), mp 175°. *Anal.* Calcd. for $C_{14}H_{12}O_3N_2$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.69; H, 4.91; N, 11.00.

Alkali Hydrolysis of IIb——A mixture of 1.0 g of IIb, 15 ml of 30% NaOH and 15 ml of glycerine was refluxed for 1 hr. After cooling, the reaction mixture was diluted with 100 ml of water and extracted with ether. The ether extract gave reddish yellow oily liquid (III), yield 0.15 g. The extraction residue was acidified with HCl, and was extracted with ether, and the solvent was removed after drying over anhyd. Na_2SO_4. The ether extract thus obtained gave a crystalline, and was recrystallized from water to yellowish white crystalline, mp 147—148° (IV). Yield 0.2 g. *Anal.* Calcd. for $C_7H_5O_4N$: C, 50.31; H, 3.02; N, 8.38. Found: C, 50.45; H, 3.17; N, 8.26.

Acetylation of III——A mixture of 0.1 g of III, 2 ml of Ac_2O and 5 ml of anhyd. pyridine was allowed to stand overnight at room temperature. The crystalline product (V) was obtained by pouring the reaction mixture into 15 ml of ice water, and was recrystallized from water to white needles, mp108°, yield 0.12 g. *Anal.* Calcd. for $C_9H_{11}ON$: C, 72.41; H, 7.37; N, 9.39. Found: C, 72.49; H, 7.33; N, 9.08.

Synthesis of 2-Nitrobenzo-*o*-toluidide (VIII)[19]——A solution of 3.5 g of *o*-nitrobenzoyl chloride dissolved in 10 ml of ether was added in a mixtur of 5 g, *o*-toluidine and 25 ml, anhyd. pyridine. The reaction mixture was allowed to stand at room temperature for 5 hr, and then it was poured into ice water. Recystallization from ethanol gave pale yellow needles, mp 175°, yield. 5.7 g. *Anal.* Calcd. for $C_{14}H_{12}O_3N_2$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.77; H, 4.74; N, 11.10.

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Results

Paper Chromatograms of Urine Extracts

The spots detected are shown in Table I.

Table I. Paper Chromatogram of Urine Extract

Spot No.	*Rf* range	From acidified urine				From basified urine			
		Dr.	I.	Eh.	Fl.	Dr.	I.	Eh.	Fl.
1	0.11—0.14	−	−	−	−	++	+	−	−
2	0.28—0.29	−	±	±	−	−	−	−	−
3	0.36—0.40	−	±	−	−	++	+	−	−
4	0.45—0.61	−	−	−	−	++	+	−	−
5	0.70—0.75	+	−	−	−	++	+	−	−
6	0.86—0.88	++	+	−	−	±	+	−	−
7	0.90—0.93	+	±	−	−	−	−	−	−
MTQ	0.86—0.88	++	+	−	±				

The acidic urine extract seemed to contain unchanged MTQ other than two kinds of metabolite, while the basified urine extract contained five kinds of metabolite only. Among the spots reacted with Dragendorff reagent, one spot (Spot 7) was faint orange brown color. All of another spots were orange yellow colors. Spot 6 was identified as unchanged MTQ.

Column Chromatography of Urine Extracts

The brown oily extracts through alumina column (2×40 cm), then the column was eluted with benzene–CH_2Cl_2 (1:1) mixture, CH_2Cl_2, and EtOH successively, and each fraction of about 100—200 ml of effluents was collected as follows; Fr. I (benzene–CH_2Cl_2, 100 ml), Fr. II, Fr. III, and Fr. IV (the same solvent, 100 ml), Fr. V (CH_2Cl_2, 200 ml), Fr. VI (CH_2Cl_2–EtOH, 130 ml). The effluents were evaporated to dryness under reduced pressure. Fr. I—Fr. V contained crystalline substances which reacted with Dragendorff reagent.

Isolation of Metabolites

The crystalline obtained from Fr. I was recrystallized from ether–petroleum ether (1:1) and gave colorless needles, mp 56—57°. The crystalline from Fr. II was recrystallized from dil. EtOH to white needles, mp 113—115°. This compound was established as unchanged MTQ by admixture with authentic sample and by its *Rf* values on paper chromatogram.

The isolation of another metabolite was carried out as follows; The solvent was removed from the effluents (Fr. III) and the yellow brown crystalline thus obtained was recrystallized from benzene to pale yellow needles, mp 175° (IIa). *Anal.* Calcd. for $C_{14}H_{12}O_3N_2$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.91; H, 4.75; N, 10.975. About 70 mg of the purified metabolite was obtained. Ultraviolet absorption spectrum of this metabolite as shown in Fig. 1 was apparently different from the spectrum of MTQ.

The infrared absorption spectrum (1523, 1354 cm^{-1}) shown in Fig. 2 suggests that this metabolite has a nitro group in its chemical structure.

After reducing the metabolite with tin dust and 4N HCl, the reduced product showed to react with Ehrlich reagent. The elemental analysis of the metabolite was coincident with that of NBT (VIII).

Rf value of this metabolite on paper chromatogram was 0.92 (Spot 7 in Table I). The metabolite is soluble in acetone, CH_2Cl_2, $CHCl_3$, and ethylacetate, but less soluble in ether and benzene.

On the other hand, IIa was confirmed to be identical with IIb which was obtained by chemical oxidation of MTQ (I).

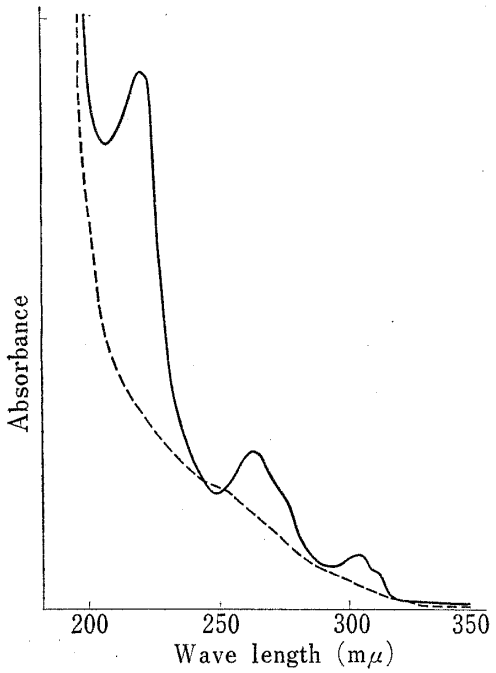

Fig. 1. Ultraviolet Absorption Spectra of MTQ (Solid Line) and Its Metabolite (Broken Line) in EtOH

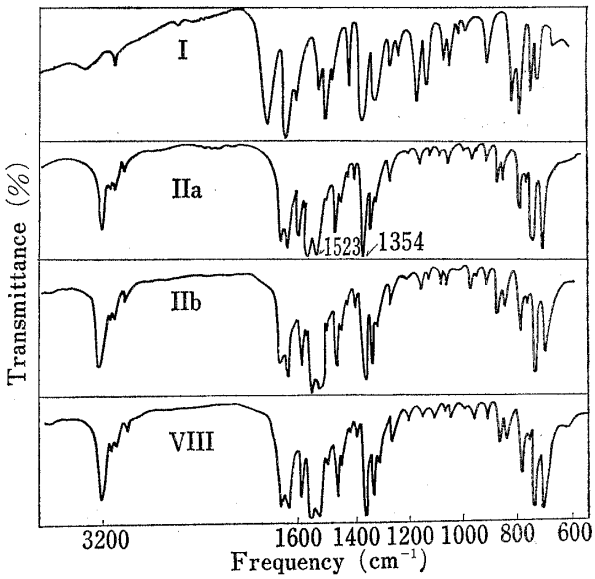

Fig. 2. Infrared Absorption Spectra of MTQ, Its Metabolite, Chemical Oxidation Product of MTQ with H_2O_2, and Synthesized 2-Nitrobenzo-*o*-toluidide (in KBr)

I: MTQ, IIa: its metabolite, IIb: chemical oxidation product of MTQ, VIII: 2-nitrobenzo-*o*-toluidide

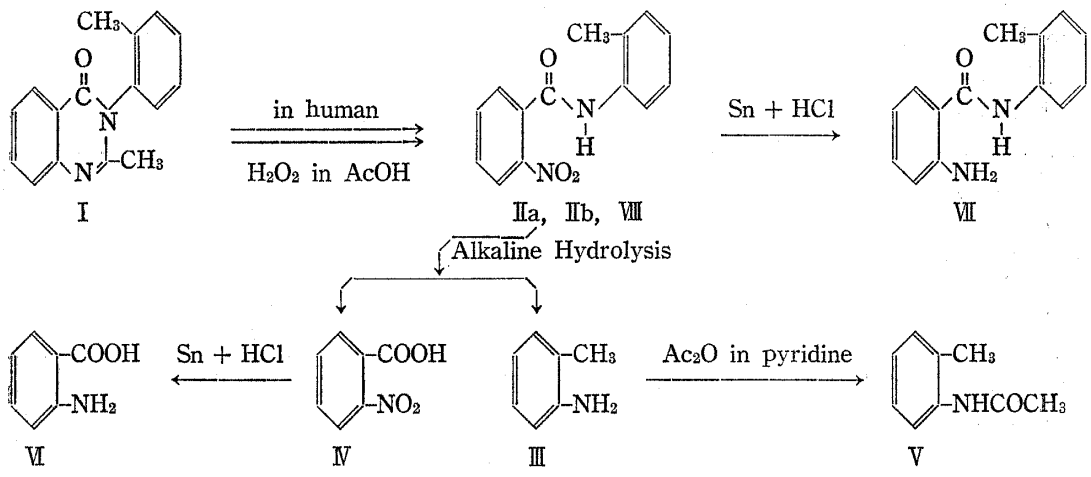

Chart 1

TABLE II. *Rf* Values of III and Reducing Products (VI, VII)

Solvent systems	*Rf* values			
	A	B	C	D
III	0.91	0.69	0.78	0.82
o-Toluidine	0.90	0.69	0.78	0.81
VI	0.39	0.84	0.87	0.84
Anthranilic acid	0.39	0.85	0.87	0.84
VII	0.89	0.96	—	—
2-Aminobenzo-*o*-toluidide	0.90	0.95	—	—

solvent systems A: BuOH saturated with 28% NH_4OH B: BuOH–AcOH–H_2O (2:1:1)
C: isopropanol–H_2O (1:1) D: MeOH–AcOH–H_2O (1:2:1)

Thus, the studies on the chemical structure of the metabolite (IIa) was carried out by using IIb as shown in Chart 1.

Alkaline hydrolysis of IIb gave *o*-toluidine (III) and *o*-nitrobenzoic acid (IV). Reduction of IV and IIb gave anthranilic acid (VI) and 2-aminobenzo-*o*-toluidide (VII), which were identified by paper chromatography as shown in Table II, respectively.

These findings suggest that IIb is 2-nitrobenzo-*o*-toluidide. Furthermore, this chemical structure of IIb was confirmed by admixture with authentic sample (VIII).

Discussion

A metabolite (IIa) of MTQ, which was isolated from the human urine by the authors, was seemed not to have any quinazolinone ring in its chemical structure from its elementary analysis. The infrared absorption spectrum and the ultraviolet absorption spectrum of the metabolite strongly suggested oxidative cleavage of the quinazolinone ring, and the former spectrum suggested that the metabolite involved a nitro group in its chemical structure. The existence of a nitro group was confirmed by reducing the metabolite with tin dust and HCl.

On the other hand, the same compound (IIb) as the metabolite IIa was obtained by the chemical oxidation of MTQ (I). And alkaline hydrolysis of the compound (IIb) gave *o*-toluidine (III) and *o*-nitrobenzoic acid (IV) as described above. Thus, both IIa and IIb were identified with the authentic 2-nitrobenzo-*o*-toluidide (VIII) by admixture and comparisons of their ultraviolet absorption spectra and infrared absorption spectra as shown in Fig. 1 and Fig. 2.

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