

## Transformation and Excretion of Drugs in Biological Systems. II.<sup>1)</sup> Transformation of Metoclopramide in Rabbits<sup>2)</sup>

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In order to understand the transformation metoclopramide, 4-amino-5-chloro-N-[2-(diethylamino)-ethyl]-2-methoxybenzamide (M), in the body, the excrements in the urine of rabbits after the oral administration were examined. As a result, the five transformation products were revealed together with the parent compound.

The urine was adjusted to pH 11—12, followed by extracting with ethylene dichloride; thus, unchanged M and the two transformation products were found in the organic layer: One of the transformates was an oxidation product of the primary amino group so that the reduction of this product resulted in the parent compound. The other was identified to be 4-amino-5-chloro-N-[2-(ethylamino)-ethyl]-2-methoxybenzamide corresponding to M de-ethylated.

At pH 4—5, a compound which was preferably extracted into ethylene dichloride was confirmed as 4-amino-5-chloro-2-methoxy benzoic acid resulting from rupture of the acid amide bond.

Water-soluble products, on the other hand, were established as N<sup>4</sup>-glucuronide and N<sup>4</sup>-sulfonate of M. These two conjugates and unchanged M were detected to be the major urinary constituents on the thin-layer chromatogram. However, no evidence was obtained for the presence of N<sup>4</sup>-acetyl conjugate.

An understanding of the fate of drugs in the body may yield valuable information in the search for more available pharmaceutical products.

Metoclopramide, 4-amino-5-chloro-N-[2-(diethylamino)-ethyl]-2-methoxybenzamide, is an antiemetic.<sup>4)</sup> In addition, Ramos, *et al.*<sup>5)</sup> pointed out that this drug exhibited an antiarrhythmic effect as well as procaine amide possessing the analogous structure. Although procaine also has the same effect, it is transient in comparison with that of procaine amide. The explanation for the difference in the activity between procaine and procaine amide is found in the relative rates of hydrolysis *in vivo* which is rapid in the former (ester link) and slow in the latter (amide link).<sup>6)</sup>

In the case of metoclopramide, Hucker, *et al.*<sup>7)</sup> described that the unchanged form in 0—24 hour urine after intramuscular administration of the drug to men represented 24% of the dose. Hitherto, however, its transformation in the body has not been investigated in detail.

Such being the case in the present paper, in order to deepen the knowledge of the transformation of metoclopramide in the body, the excrements in the urine of rabbits receiving metoclopramide dose are examined by means of thin-layer chromatography and some instrumental measurements mainly. Thus, the unchanged form and the five transformation products are presented.

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- 5) A.O. Ramos, W.P. Bastos, and M. Sakate, *Med. Pharmacol. Exp.*, **17**, 385 (1967) [*C.A.*, **68**, 76836b (1968)].
- 6) R.T. Williams, "Detoxication Mechanisms," 2nd ed., Chapman Hall Ltd., London, 1959, p. 452.
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### Experimental

All melting points were uncorrected. Infrared (IR) spectra were determined with a Shimadzu IR-A infrared spectrophotometer. Nuclear magnetic resonance (NMR) spectra were taken on a Hitachi H-60 NMR spectrometer in  $\text{CDCl}_3$  with  $\text{Me}_4\text{Si}$  as an internal standard. Mass spectra were measured with a Hitachi RMU-6E mass spectrometer.

**Metoclopramide**—The precipitate from aqueous alkaline solution of the hydrochloride obtained commercially was purified by recrystallization from benzene, mp  $146.5^\circ$ . IR  $\text{cm}^{-1}$  (Nujol):  $\nu_{\text{NH}}$  3480, 3350, 3210;  $\delta_{\text{NH}_2}$  1635; amide I band 1650, II band 1540.

**Metoclopramide-N<sup>4</sup>-glucuronide**—A mixture of 10 g of metoclopramide and 13 g of glucuronic acid in 100 ml of water was allowed to stand overnight at room temperature. Then, the reaction mixture was brought to pH 11–12 with 1N NaOH was shaken with ethylene dichloride. To the aqueous layer about 800 ml of EtOH was added and, after removal of precipitate (sodium glucuronate), the filtrate was evaporated to dryness *in vacuo* below  $60^\circ$ . The residue was recrystallized repeatedly from anhyd. MeOH<sup>8)</sup> to colorless needles, mp  $142\text{--}145^\circ$  (decomp.). Yield, 0.5 g. *Anal.* Calcd. for  $\text{C}_{20}\text{H}_{30}\text{O}_8\text{N}_3\text{Cl}$ : C, 50.47; H, 6.35; N, 8.83. Found: C, 50.38; H, 6.44; N, 8.94. IR  $\text{cm}^{-1}$  (Nujol):  $\nu_{\text{OH}}$ ,  $\nu_{\text{NH}}$  3600–3200;  $\nu_{\text{CO}}$  1610 (sh); amide I band 1640, II band 1540.

**Potassium Metoclopramide-N<sup>4</sup>-sulfonate**—To 20 ml of anhyd. pyridine 2 g of  $\text{ClSO}_3\text{H}$  was slowly added with stirring below  $15^\circ$ , and to the mixture 2 g of metoclopramide was added. After standing at room temperature overnight with occasional stirring, the reaction mixture was brought to pH 11–12 with 1N KOH followed by filtration. The filtrate was concentrated *in vacuo* and was allowed to stand overnight in a refrigerator. A white precipitate produced was recrystallized from ethanol–benzene (1:1) to leaflets, mp  $216\text{--}218^\circ$  (decomp.). Yield, 0.8 g. *Anal.* Calcd. for  $\text{C}_{14}\text{H}_{21}\text{O}_5\text{N}_3\text{ClK}$ : C, 40.23; H, 5.06; N, 10.05. Found: C, 40.28; H, 5.09; N, 10.15. IR  $\text{cm}^{-1}$  (Nujol):  $\nu_{\text{NH}}$  3400, 3300; amide I band 1640, II band 1530;  $\nu_{\text{SO}_3^-}$  1210.

**4-Amino-5-chloro-2-methoxy Benzoic Acid**—Methyl-(4-acetamido-5-chloro-2-methoxy)-benzoate was hydrolysed for 30 min at  $100^\circ$  in 2N NaOH. After adjusting to pH 2–3 with conc. HCl, the resulting precipitate was recrystallized from  $\text{CHCl}_3$  to colorless needles, mp  $205^\circ$ . *Anal.* Calcd. for  $\text{C}_8\text{H}_8\text{O}_3\text{NCl}$ : C, 47.66; H, 4.00; N, 6.95. Found: C, 47.76; H, 4.10; N, 6.89. IR  $\text{cm}^{-1}$  (Nujol):  $\nu_{\text{NH}}$  3430, 3290;  $\nu_{\text{OH}}$  2750–2650;  $\nu_{\text{CO}}$  1705;  $\delta_{\text{NH}_2}$  1645.

**N<sup>4</sup>-Acetyl Metoclopramide**—According to the conventional procedure,<sup>9)</sup> one drop of conc.  $\text{H}_2\text{SO}_4$  was added to a mixture of metoclopramide and acetic anhydride. After 10 min the reaction mixture was poured into ice water, and the resulting solution was alkalinized with  $\text{NH}_4\text{OH}$ , followed by shaking with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  layer was evaporated to dryness and the residue was recrystallized from benzene–petroleum ether (1:1) to colorless needles, mp  $101^\circ$ . *Anal.* Calcd. for  $\text{C}_{16}\text{H}_{24}\text{O}_3\text{N}_3\text{Cl}$ : C, 56.22; H, 7.08; N, 12.29. Found: C, 56.11; H, 7.21; N, 12.29.

**Thin-Layer Chromatography**—The chromatography was performed by ascending method on silica gel (Kieselgel HF<sub>254</sub> “Merck”; 0.5 mm in thickness, activated at  $110^\circ$  for 1 hr) with the solvent systems indicated in Table I.

TABLE I. Solvent Systems for Thin-Layer Chromatography

System	Contents	Ratios
A	Chloroform–methanol–28% ammonia	10:4:1
B	Propanol–28% ammonia–water	10:1:1
C	Chloroform–methanol–dioxane–28% ammonia	90:14:10:3
D	Ethylene dichloride–ethanol–28% ammonia	70:15:2
E	Chloroform–methanol–28% ammonia	70:15:2

Spray reagents used were prepared as follows: Ehrlich's reagent: 2 g *p*-dimethylaminobenzaldehyde in 25 ml conc. HCl and 75 ml MeOH. Dragendorff reagent: Prepared according to Munier modification.<sup>10)</sup> Naphthoresorcinol reagent: 2 g naphthoresorcinol in 100 ml of 20%  $\text{H}_2\text{SO}_4$ –2% EtOH (1:1).

**Animals**—Adult male albino rabbits weighing 2.5 to 3.5 kg were used.

**Preparation of Urine Sample**—The urine of rabbits receiving orally 0.1 g per kg of metoclopramide was collected for 48 hr and concentrated to a small volume *in vacuo* at  $40\text{--}50^\circ$  to apply on thin-layer plate

8) The presence of water in methanol resulted in a hydrate of the compound.

9) H. Kubota, “Zikken Kagaku Koza,” Vol. 16, ed., by Nippon Kagaku-kai, Maruzen, Tokyo, 1959, p. 294.

10) R. Munier, *Bull. Soc. Chim. Biol.*, **35**, 1225 (1953).

(untreated urine in Fig. 1). On the other hand, the urine was treated as described in Chart 1: The urine was adjusted to pH 11–12 and extracted with ethylene dichloride (EDC). The organic layer (EDC layer) was separated from the alkaline layer (aq. layer 1) and concentrated *in vacuo*. A portion of the alkaline layer was divided into two parts, and both the solutions were brought to about pH 1 by the addition of 5N HCl. After one of them was hydrolyzed for 30 min at room temperature and the other at 100°, each solution was re-adjusted to pH 11–12 (aq. layers 2 and 3). Every aqueous layer was carefully concentrated to a proper volume *in vacuo* at 40–50°.

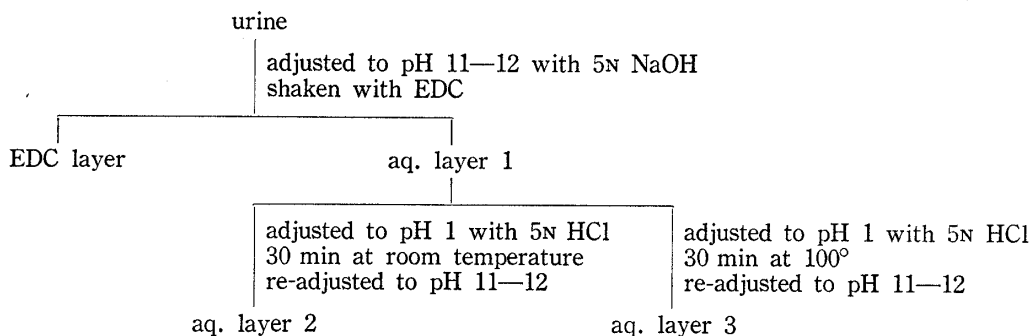


Chart 1. Treatment of Rabbit Urine receiving Metoclopramide orally for Thin-Layer Chromatographic Examination

EDC: ethylene dichloride

## Result and Discussion

### Thin-Layer Chromatography for Urine Sample

The chromatogram of rabbit urine receiving metoclopramide developed with solvent A (Table I) is shown as U, untreated urine, in Fig. 1. Six spots, on referring to the case of normal urine, were visualized by applying the spray reagents and by examination under ultraviolet light, and designated I, II, III, IV, V, and VI, respectively. From the qualitative observation for these spots, II, IV, and VI of them are supposed to be the major excrements in metoclopramide urine. All spots except I gave a positive reaction with Ehrlich's reagent, though in the cases of IV and VI the yellow color did not develop immediately after spraying.

Fig. 1 contains also the chromatogram for each sample prepared by the procedure in Chart 1. From the diagram it is obvious that I, II, and III are extracted into ethylene dichloride from metoclopramide urine alkalinized ( $U_a$ ) and the others, IV, V, and VI, remain in the aqueous layer ( $U_b$ ). By acidifying the latter, moreover, it is observed that under the mild conditions ( $U_c$ ) only VI disappears followed by appearance of II, and that under the rigorous ones ( $U_d$ ) both IV and V also do followed by appearance of II and new spot, V', which seems to be a degradation product of V as will be described later.

### Isolation of I, II, and III

Each of these spots was isolated according to the procedure diagrammed in Chart 2. Metoclopramide urine adjusted to pH 11–12 was extracted with ethylene dichloride. The

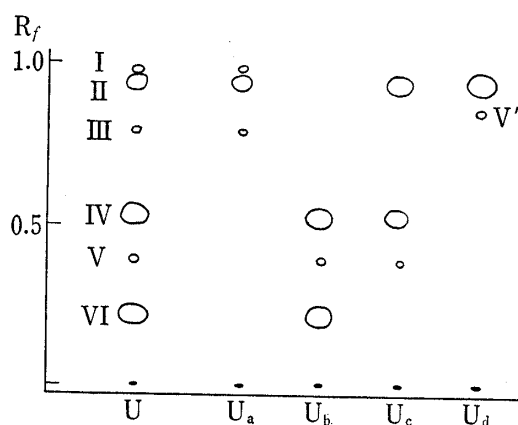


Fig. 1. Thin-Layer Chromatogram of Metoclopramide Urine untreated and treated with the Procedure in Chart 1

U: untreated urine

$U_a$ : EDC layer

$U_b$ : aq. layer 1

$U_c$ : aq. layer 2

$U_d$ : aq. layer 3

developing solvent: system A (see Table I)

All spots were detected under UV light and by applying the spray reagents (see Table II and III).

organic extract was concentrated *in vacuo* and preparatively applied on the thin-layer plate of silica gel on referring each spot crudely isolated by chromatography using solvent A. After developing with solvent C, three zones which correspond to each of I, II, and III on the plate were separately collected by scratching under ultraviolet light and eluted with solvent A (eluates I<sub>a</sub>, II<sub>a</sub>, and III<sub>a</sub>). Then, each eluate was developed with solvent D, and the scraped silica gel was again eluted with solvent A (eluates I<sub>b</sub>, II<sub>b</sub>, and III<sub>b</sub>). Eluate III<sub>b</sub> of them, moreover, was chromatographed with solvent E to obtain eluate III<sub>c</sub>. These eluates I<sub>b</sub>, II<sub>b</sub>, and III<sub>c</sub> were evaporated to dryness. On thin-layer chromatogram with solvents C, D, and E, the *R<sub>f</sub>* values and the color reactions of related substances are shown in Table II.

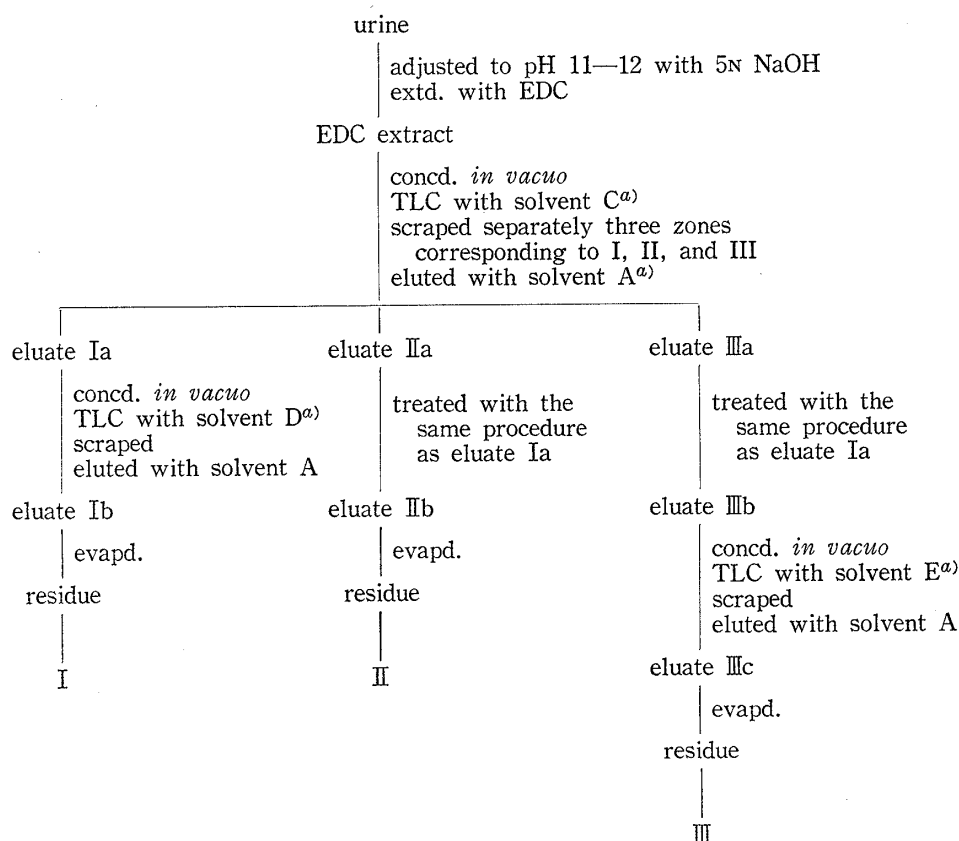


Chart 2. Isolation of I, II, and III

EDC: ethylene dichloride

<sup>a)</sup> See Table I for composition of solvents and Table II for *R<sub>f</sub>* values of related compounds in the solvents.

TABLE II. Chromatographic Properties and Color Reactions of I, II, and III

	<i>R<sub>f</sub></i> in Solvent <sup>a)</sup>				Color reaction	
	A	C	D	E	Ehrlich's reagent	Dragendorff reagent
I	0.98	0.95	0.75		—	+
II	0.94	0.68	0.48		+	+
III	0.79	0.33	0.25	0.45	+	+

<sup>a)</sup> See Table I for composition of solvents.

### Identification of I

Attempts to crystallize residue from eluate I<sub>b</sub> were unsuccessful. It, however, showed a single spot on thin-layer chromatogram and was characterized by no color reaction with

Ehrlich's reagent. While, on reducing this residue with zinc and hydrochloric acid, that the resulting product, which developed yellow color with Ehrlich's reagent, is metoclopramide was clearly demonstrated from the infrared spectrum. These facts suggest probably that I is what the primary amino group of metoclopramide was oxidized to, for example, hydroxyl-amino or nitroso group.

On the other hand, an attempt was made to oxidize metoclopramide using hydrogen peroxide in 2*N* hydrochloric acid; thus, reaction mixture was alkalinized and extracted with ethylene dichloride. As a result, among several spots on thin-layer chromatogram of the organic extract was found the spot which exhibits the same behaviors as those of I. Also in this case, because of lack in stability, it was failed to crystallize.

### Identification of II

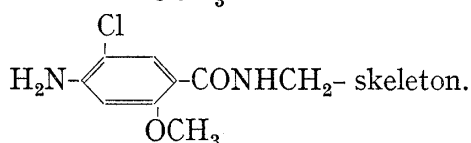
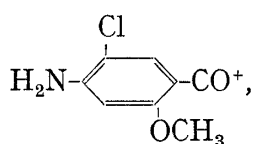
Residual solid from eluate II<sub>b</sub> was recrystallized from benzene to colorless needles, mp 148°. This compound developed yellow color promptly by spraying with the Ehrlich's reagent and orange color with Dragendorff reagent. No melting point depression was observed on admixture with metoclopramide and identity was also demonstrated by comparison of the infrared spectra. From these facts, II was confirmed as unchanged metoclopramide.

### Identification of III

The compound which was recrystallized from benzene-*n*-hexane (1:1) to colorless needles, mp 145.5—146.5°, gave a yellow color reaction with Ehrlich's reagent and a positive response in the Beilstein test.

In the infrared spectrum, the absorption bands which are considered to be due to both the primary amino and the acid amido groups were observed (IR cm<sup>-1</sup> (KBr):  $\nu_{\text{NH}}$  3500, 3350, 3200; amide I band 1640, II band 1540). Moreover, in the mass spectrum this showed

fragments at  $m/e$  213 and 184 understandable as ions  $\text{H}_2\text{N}-\text{C}_6\text{H}_3(\text{Cl})(\text{OCH}_3)-\text{CONHCH}_2^+$  and



In addition to above observations, nuclear magnetic resonance (NMR) spectra suggest that III is the N-deethylation product of metoclopramide as shown in Fig. 2: In the case of metoclopramide, singlet at 3.94 ppm is obviously due to OCH<sub>3</sub> proton. Therefore, triplet at 1.11 ppm is assigned to six protons of H<sub>a,b</sub>, and the peaks at 2.5 to 2.9 ppm and at 3.59 ppm (quartet) to H<sub>c,d,e</sub> (6H) and H<sub>f</sub> (2H), respectively. On the other hand, in the spectrum of III, although singlet at 3.94 ppm is attributed to OCH<sub>3</sub> proton similar to metoclopramide, the triplet at 1.20 ppm is equivalent to three protons and may be assigned H<sub>a</sub>. Moreover, it may be pointed out that a group of several peaks at 2.6 to 3.1 ppm are due to H<sub>c,e</sub> (4H), and that those at 3.4 to 3.8 ppm due to H<sub>f</sub> (2H).

Thus, III was confirmed as 4-amino-5-chloro-N-[2-(ethylamino)-ethyl]-2-methoxybenzamide. *Anal.* Calcd. for C<sub>12</sub>H<sub>18</sub>O<sub>2</sub>N<sub>3</sub>Cl: C, 53.04; H, 6.68. Found: C, 53.15; H, 6.64.

### Identification of IV, V, and VI

These excrements were not extracted from the urine adjusted pH 11—12 into ethylene dichloride and were relatively unstable at the acid pH as above mentioned. Accordingly,

the separation of these substances from water-soluble components existing normally in urine was difficult. Such being the case, each of IV, V, and VI was identified on referring to the standard substance as follows.

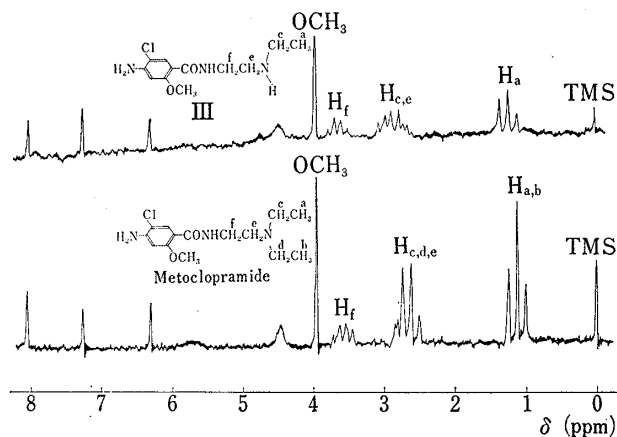


Fig. 2. Nuclear Magnetic Resonance Spectra of Metoclopramide and III in  $\text{CDCl}_3$

IV did not show a color reaction immediately by spraying the Ehrlich's reagent but after about 30 minutes it developed yellow color, and its acid hydrolysate showed the same chromatographic properties as metoclopramide. This compound, therefore, is supposed to be metoclopramide- $\text{N}^4$ -conjugate such as the sulfonate or acetylate which is stable in comparison with VI ( $\text{N}^4$ -glucuronide) described below. In this case, however, identifying as the acetylate is obviously unsuitable because of the chromatographic behaviors entirely different to synthesized  $\text{N}^4$ -acetyl metoclopramide. Whereas,

synthesized potassium metoclopramide- $\text{N}^4$ -sulfonate showed a good agreement as IV concerning the  $R_f$  values and the color reactions with spray reagents (Table III). From these facts IV was identified as metoclopramide- $\text{N}^4$ -sulfonate.

TABLE III. Chromatographic Properties and Color Reactions of IV, V, and VI

	$R_f$ in solvent <sup>a)</sup>			Color reaction	
	A	B	C	Ehrlich's reagent	Naphthoresorcinol
IV	0.53	0.37	0.10	+ (30 min after spraying)	—
V	0.39	0.31		+ (immediately)	—
VI	0.22	0.13		+ (1 min after spraying)	+

a) See Table I for composition of solvents.

VI was detected after about one minute by spraying the Ehrlich's reagent on thin-layer chromatogram, and on heating at  $60\text{--}70^\circ$  after sprayed the naphthoresorcinol reagent an intense blue appeared. While, the acid hydrolysate was identified as metoclopramide from its  $R_f$  value and color reactions. These properties and the  $R_f$  value (Table III) corresponded with those of synthesized metoclopramide- $\text{N}^4$ -glucuronide. Thus, VI was established as metoclopramide- $\text{N}^4$ -glucuronide.

V was extracted in some extent with ethylene dichloride at pH 4–5. Accordingly, it will be inferred that the compound is a relatively weak acidic substance. In this case, a benzoic acid derivative resulting from rupture of acid amide bond in metoclopramide may be taken into consideration as one of the possible structures. Thus, the  $R_f$  value and color reactions for authentic 4-amino-5-chloro-2-methoxy benzoic acid were identical to those of V (Table III). Also, treatment of authentic sample under the conditions of pH 1 and  $100^\circ$  gave a substance of the  $R_f$  value similar to V' shown in Fig. 1. From these results, V was confirmed as 4-amino-5-chloro-2-methoxy benzoic acid.

### Conclusion

In the urine of rabbits receiving metoclopramide, it was revealed by the present investigation that at least five transformation products of metoclopramide occur, together with

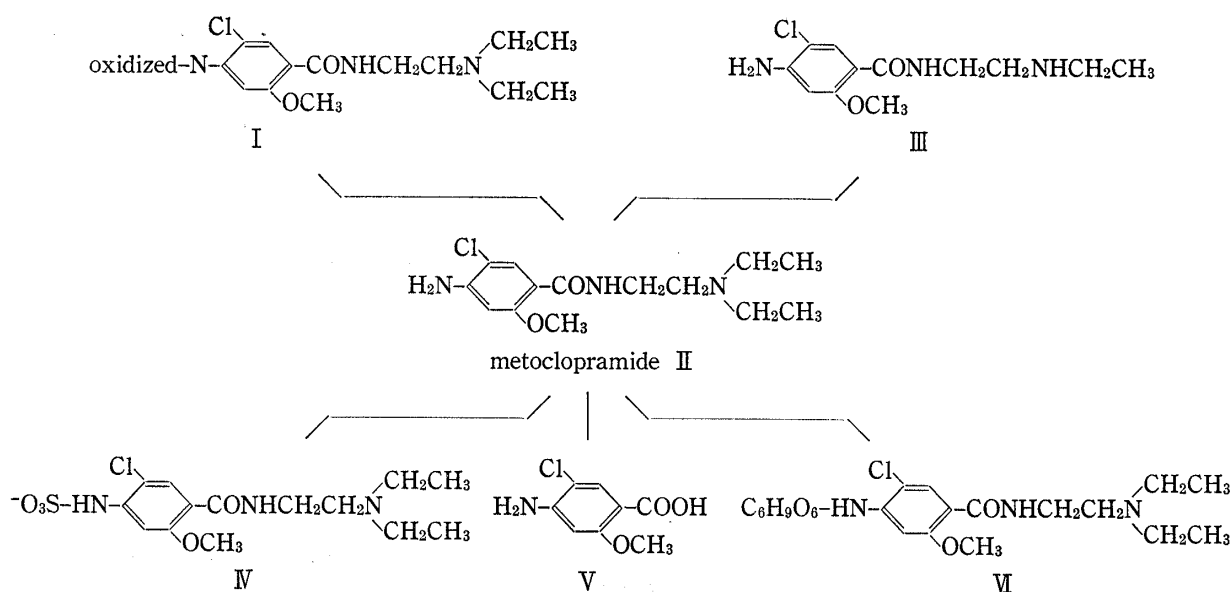


Chart 3. Transformation Products of Metoclopramide in Rabbit Urine

the parent compound, as shown in Chart 3. Of these, on the basis of the preliminary observations on thin-layer chromatogram, three excrements of unchanged metoclopramide (II) and its N<sup>4</sup>-glucuronide (VI) and N<sup>4</sup>-sulfonate (IV) are thought to be likely the major urinary constituents. While, in no case was evidence for the presence of the N<sup>4</sup>-acetyl conjugate of metoclopramide in spite of some examinations referred to the authentic compound.

In regard to procaine amide possessing the side chain similar to metoclopramide, Mark, *et al.*<sup>11)</sup> reported that a small proportion of the dose can be recovered in the urine as *p*-amino benzoic acid and its conjugates by breaking of the amide link. Similarly, in the case of metoclopramide the acid (V) being the hydrolysis product seems to be a minor urinary constituent.

An oxidation product (I) of metoclopramide was found in the urine, though attempts to specify were unsuccessful. About an oxidation of aromatic amines in animals, several workers<sup>12-14)</sup> have studied in view of the carcinogenic and the ferrihemoglobin-forming action and reported that the N-hydroxylation product or the nitroso compound was detected in the blood or urine of the animal dosed with an aromatic amine.

The metabolic N-dealkylation *in vivo* has been mainly demonstrated with the compounds possessing such N-dimethyl group as propoxyphene<sup>15)</sup> or imipramine;<sup>16)</sup> the latter is said to act through a metabolic product, desmethylimipramine.<sup>17)</sup> Thus, in the case of metoclopramide, it is interesting to note that its deethylation product (III) was found in the rabbit urine.

**Acknowledgement** The authors are indebted to the members of analytical center of Meijo University for mass measurements. Thanks are also due to Mrs. T. Tohma and Miss A. Maeda of this Faculty for elemental analyses, and to Miss Y. Kishio for NMR measurements.

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