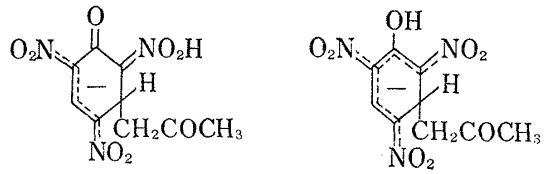

IV IV′

Chart 4

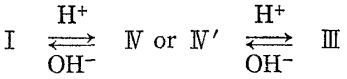

Chart 5

Experimental[15]

Preparation of III-Na——I-2Na (0.5 g) was dissolved in a mixture of MeOH (10 ml) and AcOH (3 ml). After standing for 10—12 hr at room temperature (about 25°), the solvent was removed *in vacuo* at 50°. The residue was dissolved in AcOEt (10 ml) and filtered, yellow needles were obtained. Yield, 0.28 g. mp 175° (decomp.) (from EtOH). *Anal.* Calcd. for $C_9H_8O_8N_3Na$: C, 34.95; H, 2.59; N, 13.59; Na, 7.44. Found: C, 34.95; H, 2.66; N, 13.72; Na, 7.29. $\lambda_{max}^{H_2O}$ nm (ε): 435 (19300).

Acknowledgement The authors express their gratitude to Mr. H. Matsui and Miss Y. Soeda for the IR spectral measurement, to Mr. K. Ishimura and Miss Y. Kanzaki for the elemental analysis and to Mr. Y. Tanaka for the NMR spectral measurement.

15) UV absorption spectra were measured by a Hitachi 124 Spectrophotometer equipped with a Hitachi 056 Recorder in a cell of 10 mm optical length. IR spectra were measured by a Nihonbunko DS-701G Infrared Spectrophotometer in KBr pellet. NMR spectra were measured by a JEOL C-60H NMR Spectrometer using tetramethylsilane (TMS) as an internal standard.

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Metabolism of Drugs. LXXXI.[1] Further Studies on Metabolism of Prolintane in Rabbits

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In the previous paper[3] we reported the *in vivo* metabolism of prolintane, 1-phenyl-2-pyrrolidinopentane hydrochloride in rabbits after oral administration. Rabbits receiving the drug excreted seven metabolites together with a trace amount of unchanged compound in the 24 hr urine. These were shown to be oxoprolintane, *p*-hydroxyprolintane, (ω-1)-hydroxyprolintane, *p*-hydroxyoxoprolintane, diastereoisomeric two (ω-1)-hydroxyoxoprolintanes and amino acid derivative (PPGABA).[4] On the basis of quantitative analysis, it was

1) Part LXXX: K. Tatsumi, T. Yamaguchi and H. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **21**, 622 (1973).
2) Location: *Katakasu, Higashi-ku, Fukuoka.*
3) S. Yoshihara and H. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **20**, 1906 (1972).
4) Abbreviations used are as follows; oxoprolintane: 1-phenyl-2-(2-oxopyrrolidino)pentane; *p*-hydroxyprolintane: 1-(4-hydroxyphenyl)-2-pyrrolidinopentane; (ω-1)-hydroxyprolintane: 1-phenyl-2-pyrrolidino-4-hydroxypentane; *p*-hydroxyoxoprolintane: 1-(4-hydroxyphenyl)-2-(2-oxopyrrolidino) pentane; (ω-1)-hydroxyoxoprolintane: 1-phenyl-2-(2-oxopyrrolidino)-4-hydroxypentane; PPGABA; N-(1-phenyl-2-pentyl)-γ-aminobutyric acid.

concluded that the predominant metabolites were oxoprolintane and *p*-hydroxyprolintane. Although in these experiments all of the metabolites was isolated by continuous extraction with $CHCl_3$, it was shown in the subsequent study that this continuous extraction procedure caused the non-enzymatic conversion of PPGABA to oxoprolintane.

In the present paper, the reinvestigation of the excretion pattern of urinary metabolites in rabbits and the artificial conversion of PPGABA to oxoprolintane will be reported in detail.

Experimental

Animal Experiments——Uniformly labeled ^{3}H-prolintane hydrochloride of 8.92 μCi and 8.54 μCi were administered orally in a dose of 100 mg/kg to two male albino rabbits weighing 2.7 kg (A) and 3.6 kg (B), respectively. The urine was collected using Nélaton's catheter at 1, 3, 6, 9, 12 and 24 hr after administration. The collected urine samples were filtered through hyflo supercel and adjusted to pH 9.5 with 30% NaOH.

Extraction of Metabolites——Each of the urine samples of rabbits (A) and (B) collected during the times of 0—3, 3—6 and 6—24 hr after medication was concentrated to 50 ml under reduced pressure prior to extraction. Nonconjugated metabolites in the urine were firstly extracted with the same volume of $CHCl_3$ using mechanical shaker for 20 min. The urine was then hydrolyzed with a half volume of conc. HCl and the metabolites generated from the conjugated forms were extracted with $CHCl_3$ similarly as above. From the result of recovery tests, it was shown that all other metabolites except PPGABA were extracted completely by this method. Since PPGABA is quite a polar metabolite with zwitterion structure, it could not be extracted by above shaking method, but by continuous extraction with $CHCl_3$ for 40 hr as described previously.[3] In this procedure, however, PPGABA was converted to oxoprolintane artificially, and therefore extraction and determination of PPGABA (as oxoprolintane) should be performed after all other metabolites were extracted by shaking.

Radioactivity Measurements——A Packard Tri-carb 3375 Liquid Scintillation Counter was used for the measurement of radioactivity. Radioactivities of the urine samples and extracts were measured using a toluene scintillator containing 0.4% PPO and 0.01% POPOP with or without 5% BBS-3, respectively, as described previously.[5]

Gas-Liquid Chromatography (GLC)——The instrument used was a Shimadzu Model GC-1C Gas Chromatograph equipped with hydrogen flame ionization detector. Quantitative determination of metabolites was performed following the conditions as described previously.[3] PPGABA in the urine was determined as oxoprolintane converted from PPGABA during continuous extraction procedure.

Thin-Layer Chromatography (TLC)——Thin-layer plates of silica gel (Kieselgel G, Merck; 0.25 mm thick, activated at 105° for 30 min) were used in the present experiments. Solvent system used was benzene–acetone–MeOH (7 : 1 : 2). Dragendorff reagent was used for detection of metabolites as described previously.[3]

Result and Discussion

As shown in Fig. 1, rabbits given ^{3}H-prolintane excreted about 70% of radioactivity in the 24 hr urine, most of which was found in the urine during the first 6—9 hr. These results suggested that the excretion of metabolites was considerably rapid in rabbits.

In these experiments the extraction of metabolites was carried out by shaking. By this method all other metabolites except PPGABA was extracted completely. After extraction of hydrolyzed urine by shaking, about 30% of the radioactivity administered still remained in the urine (Fig. 1). In order to explore this unextractable metabolite(s), the urine was applied to Amberlite XAD-2 resin column chromatography as follows; 10 ml of the urine, from which all other metabolites were extracted off, was applied to 50 ml of Amberlite XAD-2 resin column and eluted with 150 ml of MeOH. MeOH eluate was concentrated to a small volume under reduced pressure and submitted to GLC and TLC. On GLC it revealed a peak at t_R 4.0 min as same as oxoprolintane, whereas on trimethylsilylation with N,O-bis-(trimethylsilyl)acetamide prior to injection, it gave a peak at t_R 5.8 min. These results suggested that the original metabolite in MeOH eluate was not oxoprolintane which could not be trimethylsilylated, but converted to oxoprolintane on GLC. In agreement with this

5) S. Yoshihara and H. Yoshimura, *Biochem. Pharmacol.*, 21, 3205 (1972).

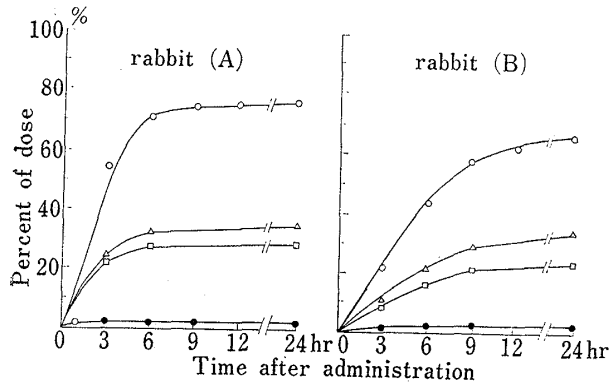

Fig. 1. Urinary Excretion of Metabolites from Rabbits Given ^{3}H-Prolintane

—○—; total radioactivity excreted in urine
—●—; radioactivity of nonconjugated metabolites
—□—; radioactivity of conjugated metabolites
—△—; radioactivity remained after shaking extraction of hydrolyzed urine

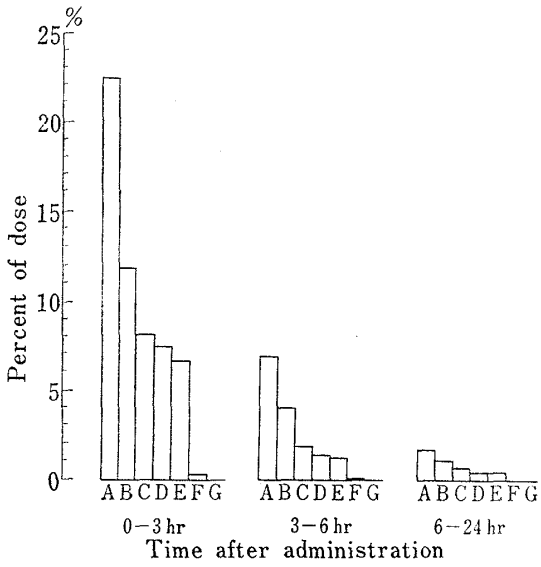

Fig. 2. Urinary Excretion Pattern of Metabolites from Rabbit (A) Given ^{3}H-Prolintane

(A) PPGABA
(B) *p*-hydroxyprolintane
(C) *p*-hydroxyoxoprolintane
(D) (ω-1)-hydroxyoxoprolintane (II)
(E) (ω-1)-hydroxyoxoprolintane (I)
(F) (ω-1)-hydroxyprolintane
(G) oxoprolintane

suggestion, the metabolite gave a spot at *Rf* 0.10 on TLC with a solvent system of benzene–acetone–MeOH (7: 1: 2), whereas oxoprolintane showed *Rf* value of 0.70. This *Rf* value of 0.10 was identical with that of previously isolated sample of PPGABA.[3] And the retention time of trimethylsilyl (TMS) derivative of PPGABA (5.8 min) was also shown to be identical with that of the TMS-metabolite.

This remained PPGABA in urine could be extracted only by continuous extraction with $CHCl_3$. However, if once extracted, it gave no longer a peak at t_R 5.8 min on GLC and a spot at *Rf* 0.10 on TLC. Instead, it gave the identical t_R (4.0 min) and *Rf* (0.70) with those of oxoprolintane on GLC and TLC, respectively.

From these results it was concluded that PPGABA was converted to oxoprolintane during continuous extraction. This artificial conversion of PPGABA to oxoprolintane was also confirmed by the same treatment of the control urine added with PPGABA.

Fig. 2 shows the urinary excretion patterns of all metabolites. From these results it was considered that the metabolism of prolintane was very fast and the excretion pattern of metabolites was similar among the three divided intervals during 24 hr. In addition it was clarified that the previous conclusion[3] that the major metabolite of prolintane was oxoprolintane was not correct, but PPGABA was actually the major metabolite.

In order to elucidate the correlation of PPGABA and oxoprolintane in prolintane metabolism, oxoprolintane was administered orally to rabbits in a dose of 33.4 mg/kg. The result showed no formation of PPGABA, but of *p*-hydroxyoxoprolintane (12.5%) and two diastereoisomeric (ω-1)-hydroxyoxoprolintanes ((I) 10.8% and (II) 7.2%), respectively.

In the next experiment, 2 μmoles of PPGABA was incubated with rabbit liver homogenate or $9000 \times g$ supernatant as same as previously described,[5] to learn whether oxoprolintane is formed enzymatically from PPGABA. None of oxoprolintane, however, could be detected in the incubation mixture.

These results strongly suggest that in prolintane metabolism PPGABA and oxoprolintane are not obligatory intermediate of each other and they are derived separately from a common intermediate, postulated as "hydroxyprolintane" (Fig. 3).

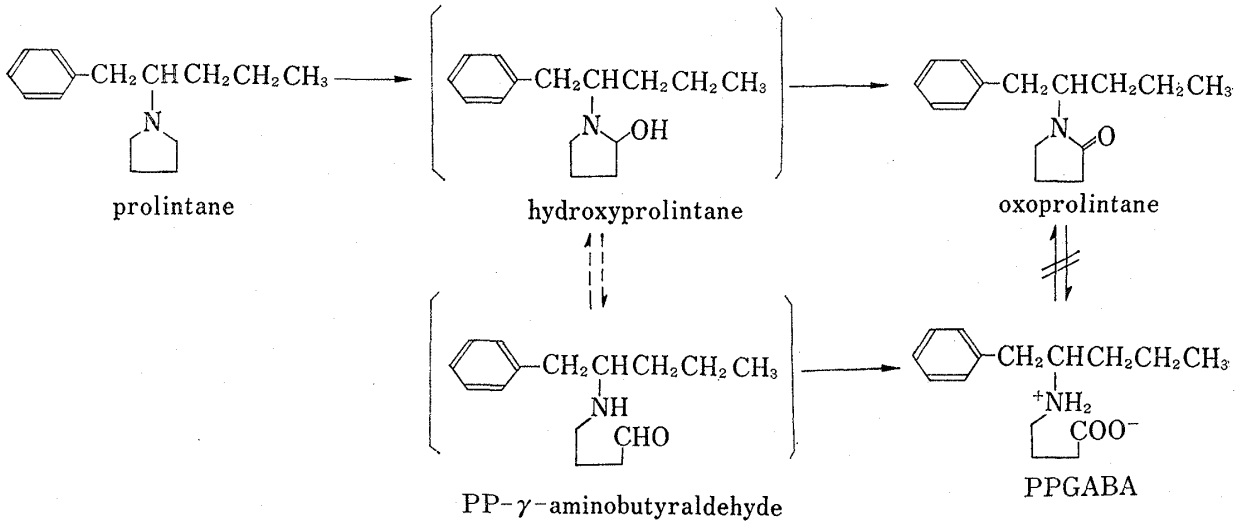

Fig. 3. The Possible Mechanism of Pyrrolidine Ring Oxidation in Rabbits Given Prolintane

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