Metabolism of a Pharmacologically Active Pyrrolidine Derivative (Prolintane) by Lactam Formation

N-dealkylation of drugs and other foreign compounds by liver microsomal enzymes is a well-known metabolic pathway. A less well studied type of N-dealkylation[1] is by oxidation of a heterocyclic compound to the corresponding lactam. Nicotine and tremorine, 1,4-*bis*-(pyrrolidino)-butyne-2, for example, are dealkylated in this manner by conversion to the corresponding pyrrolidones[2,3]. The reaction with nicotine proceeds through the α-hydroxy pseudo-base[4] while that with tremorine generates an active metabolite, oxotremorine[3]. An additional example of this type of N-dealkylation is reported here for prolintane, DL-1-(α-propylphenethyl)-pyrrolidine hydrochloride, an analeptic drug[5] (Figure 1). The metabolite was initially identified as a product of the metabolism of prolintane by rabbit liver in vitro.

Liver from a male New Zealand white rabbit was homogenized at 4 °C in 2 volumes of 0.16 *M* KCl and the 9000 × *g* supernatant fraction prepared as previously described[4]. Prolintane labelled with ^{14}C in the 2 and 5 carbons of the pyrrolidine ring (9.2 μmoles, 0.05 μci), NADP (0.5 μmole), glucose-6-phosphate (25 μmoles), nicotinamide (100 μmoles), $MgCl_2$ (75 μmoles) and phosphate buffer (pH 7.4) were incubated with 9000 × *g* supernatant equivalent to 1 g of liver (final volume = 5.0 ml) for 1.5 h at 37 °C in air. The incubation mixture was extracted with heptane containing 3% isoamyl alcohol (5 vols), which was back-extracted with 0.1 *N* HCl. Radiometric assay of the acidic solution, which contained only prolintant (as shown by TLC), revealed that approximately 85% of the added drug was metabolized. The remaining heptane contained about 65% of the added radioactivity. Thin-layer chromatography of the concentrated heptane extract revealed the presence of a single major metabolite, identical with authentic 1-(α-propylphenethyl)-pyrrolidin-2-one (Table).

The metabolite was isolated by scraping the radioactive spot off the plate and shaking with phosphate buffer (pH 7.4) and heptane. The concentrated heptane extract was subjected to gas chromatography on an F+M gas chromatograph, fitted with a 6 foot glass column, packed with 1% QF-1 on Gas Chrom Q, and a flame ionization detector, using helium as carrier gas (50 ml/min) at 150 °C. The retention time of the metabolite was identical with that of authentic 1-(α-propylphenethyl)-pyrrolidin-2-one (7.2 min). In addition, the mass spectrum of the metabolite was shown to be identical to that of authentic compound (Figure 2).

The experiment was repeated with liver from 2 additional rabbits. In each case, the lactam metabolite was identified and represented approximately 50 to 60% of the prolintane added to the incubation mixture. These results indicated that 1-(α-propylphenethyl)-pyrrolidin-2-one is a major product of the metabolism of prolintane by rabbit liver in vitro.

Although prolintane was rapidly metabolized by rabbit liver in vitro, rat liver microsomes (9000 × *g* supernatant) metabolized the drug to only a slight extent. In this respect the drug resembles amphetamine, which is extensively metabolized by rabbit liver in vitro[8] but not by rat liver[9]. Pretreatment of rats with phenobarbital (40 mg/kg, p.o., twice daily for 4 days prior to sacrifice) stimulated the metabolism of prolintane in vitro only to a small extent (+10%).

The lactam metabolite was also identified in liver of rats given prolintane-^{14}C in the following experiment. Two rats were given 10 mg/kg of labelled drug i.v. and sacrificed after 0.5 h. Livers were removed and homogenized in water. The lactam metabolite was isolated by extraction with heptane as described above. Unchanged drug was removed by back-extraction into 0.1 *N* HCl. Radioactive assay of the remaining heptane showed that the livers of each rat contained, respectively, 1.2 μg and 0.3 μg of apparent lactam metabolite per gram of liver. The metabolite was further identified by thin-layer and gas chromatography, as described in the following experiments.

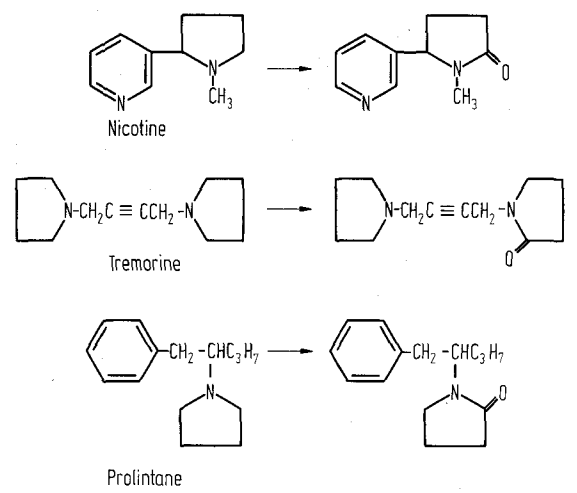

Fig. 1. Examples of N-dealkylation of N-heterocyclic compounds by oxidation to the corresponding lactam.

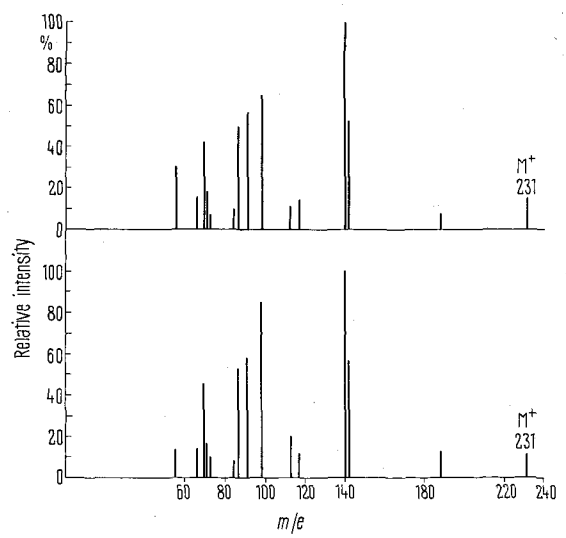

Fig. 2. Mass spectra of metabolite of prolintane (lower) and 1-(α-propylphenethyl)-pyrrolidin-2-one.

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The heptane extract of the apparent lactam metabolite was subjected to thin-layer chromatography on silica gel in several solvent systems. Only one radioactive spot was present, which corresponded in Rf value to that of the authentic lactam (Table). The radioactive material was eluted and subjected to preparative gas chromatography at 180 °C in a Perkin-Elmer gas chromatograph, equipped with 6 foot glass columns packed with 1% QF-1 on Gas Chrom Q and stream-splitter (80:20). A major peak was observed with a retention time of 2.4 min, the same as that of authentic lactam. Eluates from the column were trapped in Pasteur pipets and the contents counted. Only the peak corresponding in retention time with that of the lactam metabolite (2.4 min) contained significant radioactivity.

Rats given prolintane-^{14}C (10 mg/kg, i.v.) excreted approximately 40% of the dose in the urine in 24 h but only traces, if any as the lactam metabolite. The drug itself was almost completely metabolized.

Thin-layer chromatography of the metabolite and authetic 1-(α-propylphenethyl)-pyrrolidin-2-one

Solvent system	Metabolite	Synthetic compound
1	0.60	0.60
2	0.44	0.44
3	0.58	0.58

Glass plates coated with silica gel G, 250 μm. Compounds were detected by radiometric scanning and Dragendorf's reagent. Solvent systems were prepared as follows: 1. benzene-dioxane-ammonia 60:35:5[6]; 2. cyclohexane-diethylaminebenzene 75:20:15[7]; benzene-ethanol-12 *N* ammonia 95:15:5[7].

In conclusion, the present study describes a further example of metabolic conversion of a substituted pyrrolidine to the corresponding lactam. Since the lactam may, potentially, at least, be converted to the open-chain amino acid, the reaction is properly classified as a type of N-dealkylation. Information on the mechanisms of drug metabolism reactions may have, in addition to its theoretical value, important pharmacologic application, since the various intermediate formed may themselves posses biologic activity.

Zusammenfassung. Prolintan, N-1-(α-Benzylbutyl)-Pyrrolidin, wurde in Kaninchen durch ein Mikrosomensystem der Leber in das entsprechende Laktam, N-1-(α-Benzylbutyl)-Pyrrolidin-2-on, umgebaut. Das gleiche Stoffwechselprodukt fand sich auch im Gewebe von Ratten, denen das Medikament gegeben wurde; allenfalls Spuren davon wurden im Urin der Ratten ausgeschieden.

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A Note of the Dual Effect of Prostaglandin E_1 on the Responses of the Guinea-Pig Vas Deferens to Nerve Stimulation

Divergent results have been obtained concerning the effects of prostaglandins on the motor response of the guinea-pig vas deferens to nerve stimulation. Mantegazza and Naimzada[1] reported that high concentrations of PGE_1 potentiated this response. Sjöstrand and Swedin[2] noted that PGE_1 usually caused an inhibition but sometimes a potentiation of the response. Euler and Hedqvist[3] observed that low concentrations of PGE_1 and PGE_2 inhibited the response, while it was enhanced by higher concentrations. Ambache and Zar[4] found that low concentrations of PGE_2 inhibited the response to low frequency stimulation but rather potentiated the response to high frequency stimulation. In order to obtain a somewhat more detailed information on the action of PGE_1 on the response to nerve stimulation of the guinea-pig vas deferens, a study with combined electrical and mechanical registration has been performed.

Material and methods. For electrical recording the sucrose gap technique was used. The preparation, sucrose gap apparatus with stimulating electrodes, solutions, temperature and other conditions were essentially the same as those used by Burnstock et al.[5]. Isometric tension was recorded with a strain gauge transducer. Conventional amplifiers and a Hellige Helcoscriptor (He-86) were used. Nerve stimulation with square wave pulses of 0.5 msec duration and a voltage of 10–40 V was applied by a Grass S4 stimulator. The report is based on observations on 14 vasa deferentia from 10 guinea-pigs weighing about 500 g.

Results. PGE_1 in concentrations of 10–200 ng/ml caused a depolarization of the smooth muscle of the vas deferens. The depolarization was usually moderate as in Figure 1a. It increased in amplitude with increasing PGE_1 concentration but was never large enough to reach the threshold for firing of action potentials (AP). However, there were rather large differences in sensitivity among the preparations.

PGE_1 decreased the magnitude of evoked excitatory junction potentials (EJP) (Figures 1 and 2). The decrease in size of the EJPs was alwayr much larger than that which might be expected from the degree of depolarization. The reduction of the EJPs was seen in sensitive preparations with the lowest concentration of PGE_1 (10 ng/ml) and became more pronounced when the concentration was increased. In most preparations maximal inhibition was obtained at about 100 ng/ml of PGE_1 and a further increase in concentration of the drug added very little to the inhibition already obtained. As with the depolarizing action of PGE_1, there were rather large

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