Metabolites of Loperamide in Rats

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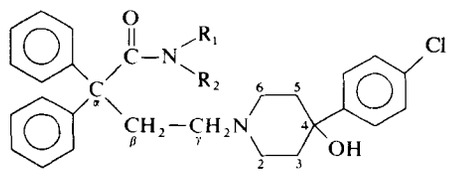
Following intraperitoneal administratin to rats of [^{14}C]loperamide, [carbonyl-^{14}C] 4-(*p*-chlorophenyl)-4-hydroxyl-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutyramide, metabolites in feces and urine were separated, and identified by means of mass spectrometry. In feces, six metabolites were identified in addition to the unchanged drug. The main metabolic pathways involved are dealkylation in the dimethyl amide moiety to give desmethyl- and didesmethylloperamide, both of which were in turn monohydroxylated either in the α-phenyl ring or possibly in the α-carbon in the piperidine ring. It is noteworthy that metabolites hydroxylated in the piperidine ring were isolated as pyridinium derivatives, possibly due to spontaneous aromatization of its 2,4-dihydroxy-4-(*p*-chlorophenyl)piperidine ring. In urine, only two metabolites were found and identified to be desmethyl- and didesmethylloperamide, since [^{14}C]loperamide was excreted into urine only in a small amount.

INTRODUCTION

Loperamide (**1**), 4-(*p*-chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutyramide hydrochloride, is a potent antidiarrhoeal agent developed recently; the pharmacology of the drug is well established.[1–3] Pharmacokinetic studies have been performed in rats and men using [^{3}H]loperamide labelled at the N—CH_3 group,[4] and in rats using [^{3}H]loperamide labelled at the adjacent position to the chlorine substitution.[5] Non-volatile metabolites and tritiated water have been found in plasma of rats and men receiving the former labelled drug, while two demethylated metabolites (**2** and **3**) and 4-(*p*-chlorophenyl)-4-hydroxypiperidine along with the unchanged drug have been



(**1**) R_1, $R_2 = CH_3$
(**2**) $R_1 = H$, $R_2 = CH_3$
(**3**) R_1, $R_2 = H$

identified in excreta of rats given the latter label. These findings suggest that loperamide is metabolized extensively in rats and men, which prompted us to perform further metabolic studies using [^{14}C]loperamide labelled at the carbonyl carbon.

This paper discusses the identification of metabolites by mass spectrometry in rat excreta after intraperitoneal administratin of [^{14}C]loperamide.

EXPERIMENTAL

Materials

4-(*p*-Chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutyramide hydrochloride (loperamide), 4-(*p*-chlorophenyl)-4-hydroxy-*N*-methyl-α,α-diphenyl-1-piperidinebutyramide hydrochloride (desmethylloperamide) and 4-(*p*-chlorophenyl)-4-hydroxy-α,α-diphenyl-1-piperidinebutyramide hydrochloride (didesmethylloperamide) were suplied from Janssen Pharmaceutica, Beerse, Belgium.

[^{14}C]Loperamide labelled at the carbonyl carbon with a specific activity of 49.5 μCi mg^{-1} was prepared essentially according to the procedure reported for loperamide.[6] Its radiochemical purity was virtually 100% as assayed by thin-layer chromatography (TLC) and inverse dilution analysis.

1-Methyl-3,3-diphenyl-2-pyrrolidinone[7] and (tetrahydro-3,3-diphenyl-2-furylidene)methylamine[6] were synthesized as described previously. 4-(*p*-Chlorophenyl)pyridine was prepared by Cope degradation of desmethylloperamide *N*-oxide which was obtained by oxidizing desmethylloperamide with *m*-chloroperbenzoic acid in methanol.

All these specimens were checked by NMR and/or MS to be pure enough as authentic samples.

Biological samples

An aqueous solution of [^{14}C]loperamide diluted with non-labelled loperamide was injected intraperitoneally to 10 male Wistar rats weighing about 250 g in a dose of 25 mg kg^{-1}. Urine and feces were collected separately for up to 72 h. Feces were homogenized in water. Radioactivity in fecal and urinary samples were determined in a Packard Tri-Carb 3380 liquid scintillation spectrometer.[8]

Fractionation and isolation of metabolites

The fecal homogenate or urine was extracted with chloroform at pH 10. The organic phase was washed with 0.1 N HCl and the aqueous phase was re-extracted into ether after adjustment to pH 10. The ether phase was evaporated to dryness and the residual radioactive material was termed as the basic fraction. The fecal homogenate or urine free portion of the basic fraction was then acidified to pH 3 and extracted with ethyl acetate. The organic phase was washed with 0.1 N

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NH_4OH. After acidification to pH 4 the aqueous phase was re-extracted with ether. The ether phase was evaporated and the residual material was termed as the acidic fraction.

Both the basic and the acidic fractions of feces or urine were subjected to TLC with the solvent system (A) chloroform + isopropanol + NH_4OH (70:30:1)[4] or (B) *n*-butanol saturated with NH_4OH, and the radioactive materials were located by scanning the TLC plate (silica gel 60 F_{254} of 0.25 mm thickness Merck, Darmstadt, Germany) with the Packard radiochromatogram scanner. The radioactive zones were separately scraped off from the TLC plate and the radioactive materials were eluted with ether or with methanol for A_7. Each eluate was concentrated and subjected to mass spectrometric analysis.

Mass spectrometry

The mass spectra were recorded on an Hitachi RMU-6L single focusing mass spectrometer using the direct inlet system. The ionization potential and trap current were 70 eV and 60 μA, respectively. A_{7a}, a metabolite, was also measured by GCMS. The analysis was performed on the same mass spectrometer equipped with an Hitachi M-52 gas chromatograph. A glass column (1 m × 3 mm) packed with 3% OV-25 on Gas Chrom Q was used. GCMS conditions were as follows: injection port temp., 300 °C; oven temp., 240 °C; carrier gas (He), 1.2 kg cm^{-2}; ionization potential, 20 eV; trap current, 50 μA; source temp., 200 °C. The field desorption (FD) mass spectrum of A_{7a} was recorded on a JEOL JMS-D100 double focusing mass spectrometer equipped with an FD ion source. The accelerating voltage and cathode voltage were 3 kV and −5 kV, respectively.

RESULTS AND DISCUSSION

Separation of metabolites

Seventy-two hours after the administration of [^{14}C]loperamide to rats, radioactivity in feces and urine accounted for 75% and 12% of the dose respectively. Fractionation of feces, the major excreta, by solvent extraction gave 58% of the fecal radioactivity in the basic fraction, 17% in the acidic fraction and the remaining 25% in the aqueous solution.

The thin-layer radiochromatogram of the fecal basic fraction gave seven peaks (R_f = 0.77, 0.65, 0.56, 0.43, 0.29, 0.18 and origin) in the solvent system (A) as shown in Fig. 1(a). Metabolites associated with peaks are designated from A_1 to A_7 (origin) in decreasing order of R_f-values. A_7 was recovered from the plate and rechromatographed in the solvent system (B) to give two peaks (A_{7a} and A_{7b}) as shown in Fig. 1(b).

On the other hand, radiochromatograms of either the acidic or aqueous fractions showed only two significant peaks corresponding to A_{7a} and A_{7b}.

Fractionation of urine, the minor excreta, resulted in 63% of the urinary radioactivity in the basic fraction and a few percent in the acidic fraction. The basic fraction alone gave two significant peaks in the radiochromatogram which corresponded to A_3 and A_4.

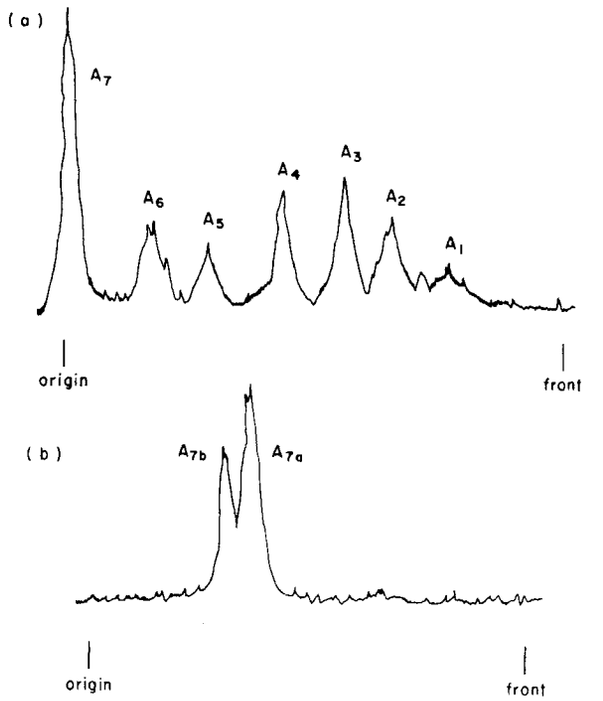


Figure 1. Radiochromatograms of (a) fecal basic fraction in solvent system (A) and (b) A_7 [origin in Fig. 1(a)] in solvent system (B).

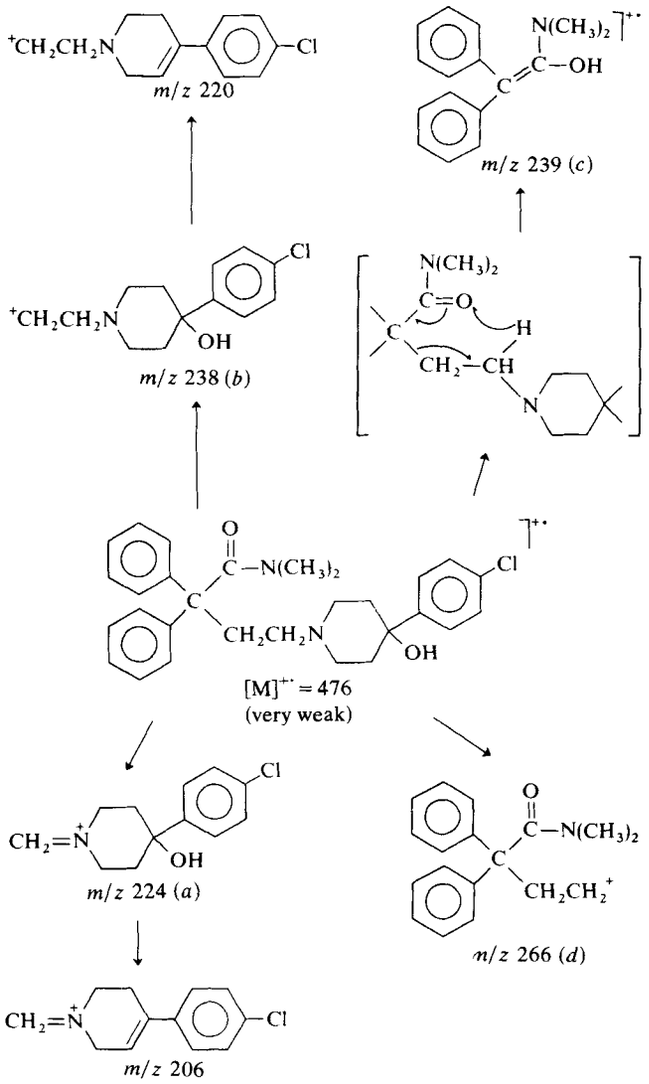
Mass spectrum of loperamide

On electron impact (EI) the mass spectrum of loperamide give three prominent peaks at *m/z* 224, 238 and 239, along with a weak peak at *m/z* 266 and a very weak peak of the molecular ion (0.2% rel. int.) as shown in Fig. 2(b). The *m/z* 224 and 238 ions, designated as ions *a* and *b* respectively, can be rationalized by simple cleavages of the C(β)—C(γ) and C(α)—C(β) bonds of the butyramide chain, respectively. The *m/z* 239 ion, designated as ion *c*, is probably formed by McLafferty rearrangement. The *m/z* 266 ion, designated as ion *d*, is formed by simple cleavage of the C—N bond between the butyramide chain and piperidine ring. Both ions *a* and *b* are associated with the 4-(*p*-chlorophenyl)-4-hydroxypiperidine moiety, while ions *c* and *d* are associated with the *N,N*-dimethyl-α,α-diphenylbutyramide moiety. The major fragmentation process of loperamide is summarized in Scheme 1.

Since *a*, *b* and *c* type ions were commonly prominent in the mass spectra of loperamide derivatives, they could be key ions facilitating an indication as to the moiety of the molecule in which the metabolic modification occurred.

Structural identification of metabolites

Preliminary gas chromatographic examination of loperamide derivatives failed to give reasonable separation even after derivatizations such as silylation with BSTFA or acylation with heptafluorobutyric anhydride. Therefore, metabolites were separated by solvent



Scheme 1. Major fragmentation process of loperamide.

extraction, followed by TLC purification as described before and subjected to mass spectrometric measurements.

Of eight fecal metabolites, A_1 did not afford a mass spectrum relevant for the structural analysis, since it was a minor metabolite and heavily contaminated with fecal components even after further purification.

The mass spectrum of A_2 was identical to that of the authentic loperamide [Fig. 2 (a and b)]. The unchanged drug was thus excreted in feces in agreement with previous findings.[4,5]

A_3 exhibited abundant peaks at m/z 224, 225 and 238 [Fig. 3(a)]. The peaks at m/z 224 and 238 correspond to ions *a* and *b*, respectively, as found for loperamide, whereas the peak at m/z 225 is smaller than the *c* ion by 14 amu, suggesting demethylation involved in A_3. A_3 also exhibited weak peaks at m/z 250 and 252; the latter peak is smaller by 14 amu than ion *d*, while the former is possibly ascribed to a peak associated with the thermal decomposition of desmethylloperamide as will be discussed for A_{7a}. The mass spectrum of the authentic desmethylloperamide was confirmed as being identical to that of A_3 [Fig. 3(b)].

The mass spectrum of A_4 [Fig. 4(a)] also gave abundant peaks at m/z 224 and 238, together with a peak at

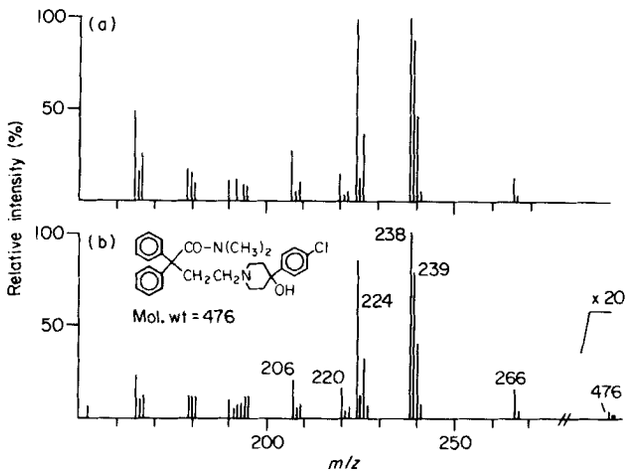


Figure 2. Mass spectra of (a) A_2 and (b) loperamide.

m/z 211 instead of the peak at m/z 225 in that of A_3. The decrease by 14 amu suggested further demethylation involved in A_4, which was confirmed by spectral identification with didesmethylloperamide [Fig. 4(a)].

These two identified metabolites indicate that loperamide undergoes sequential demethylation in the rat; this is consistent with previous findings.[5]

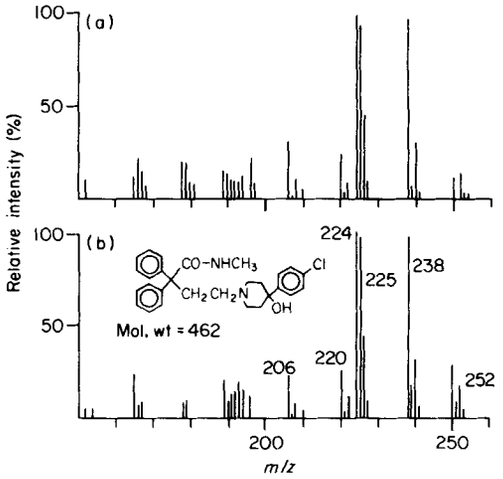


Figure 3. Mass spectra of (a) A_3 and (b) desmethyloperamide.

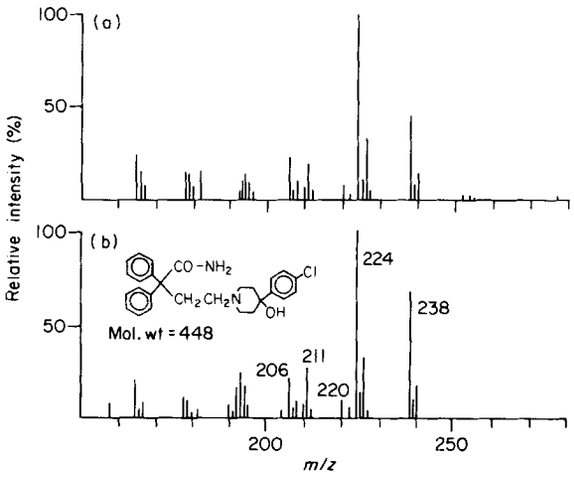


Figure 4. Mass spectra of (a) A_4 and (b) didesmethyloperamide.

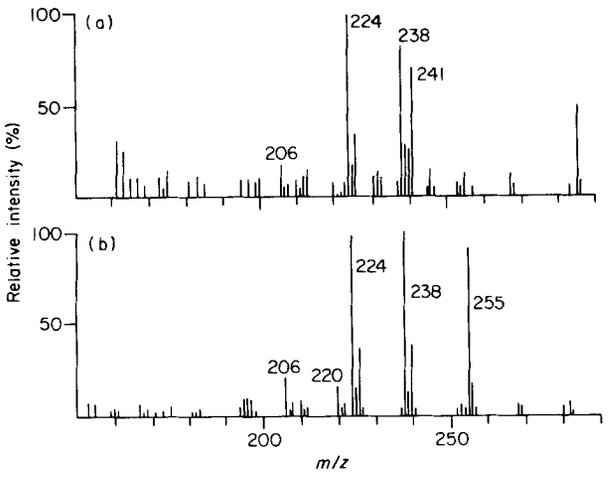


Figure 5. Mass spectra of (a) A_5 and (b) A_5 treated with diazomethane.

A_5 showed prominent peaks at m/z 224, 238 and 241 [Fig. 5(a)]. The peaks at m/z 224 and 238 correspond to the *a* and *b* type ions of desmethylloperamide, while the ion at m/z 241 corresponds to the *c* type ion (m/z 225) of desmethylloperamide with a difference of 16 amu. Since the *c* type ion involves the diphenyl group, the increase of 16 amu suggests the presence of an hydroxy group in the α-phenyl ring. In order to confirm this, A_5 was treated with diazomethane. The *c* type ion of desmethylloperamide at m/z 241 was in fact shifted by 14 amu to m/z 255, whereas the peaks at m/z 224 and 238 were unchanged as shown in Fig. 5(b). Although the increase by 14 amu in the *c* type ion might also be ascribed to methylation of the amide group, authentic desmethylloperamide was not methylated by diazomethane. Therefore, A_5 was assigned to desmethylloperamide with an α-phenyl ring hydroxylated. A_5 showed also peaks at higher masses than m/z 260, but after methylation their intensities decreased as compared with those of the key ions, suggesting that they were due to fecal contaminants.

A_6 showed abundant peaks at m/z 224, 227 and 238 [Fig. 6(a)]. The ions at m/z 224 and 238 correspond to the *a* and *b* type ions of didesmethylloperamide, while the ion at m/z 227 corresponds with a difference of 16 amu to the *c* type ion (m/z 211) of didesmethylloperamide. The presence of an hydroxyphenyl group was also confirmed by methylation of A_6. After treatment with diazomethane, a selective increase of 14 amu was found for the *c* type ion (m/z 227 to m/z 241) but not for the *a* and *b* type ions (m/z 224 and m/z 238, respectively), as shown in Fig. 6(b). Based on similar evidence found for A_5, A_6 was assigned to didesmethylloperamide with an α-phenyl ring hydroxylated.

The mass spectra of A_{7a} and A_{7b} [Fig. 7 (a and b), respectively] were similar to each other except that an ion cluster at m/z 250–251 in A_{7a} shifted by 14 amu to m/z 236–237 in A_{7b}, suggesting analogous metabolites with demethylation involved in the latter. However, both spectra lacked typical prominent peaks as found with A_2–A_6, but gave a base peak at m/z 189, suggesting different fragmentation involved in the metabolites or thermal decomposition occurring in the mass spectrometer. Since both metabolites are highly polar, as suggested by TLC, the latter possibility is likely.

When A_{7a} was subjected to GCMS, three gas chromatographic peaks (p-1 to p-3 in Fig. 8) were obtained exhibiting the molecular ion peaks at m/z 189 in p-1 and m/z 251 in both p-2 and p-3. These were identified by mass spectral comparison of authentic specimens as 4-(*p*-chlorophenyl)pyridine[p-1], (tetrahydro-3,3-diphenyl-2-furylidene)methylamine[p-2] and 1-methyl-3,3-diphenyl-2-pyrrolidinone[p-3][Fig. 9 (a, b and c), respectively]. Since the TLC properties of these three compounds differed from those of A_{7a}, thermal degradation of A_{7a} possibly occurred in the injection port of the gas chromatograph. Similarly, in the direct probe, thermal degradation of A_{7a} occurred to afford a combined mass spectrum of the above three compounds as seen in Fig. 7(a). A biologically possible metabolite, desmethylloperamide *N*-oxide, gave similar peaks at m/z 189 and 251.

To obtain the mass spectra under thermally mild conditions, the FD mass spectra of A_{7a} and desmethylloperamide *N*-oxide were obtained. A_{7a} showed a prominent peak at m/z 441 [Fig. 10(a)] corresponding to a pyridinium derivative of desmethylloperamide, while desmethylloperamide *N*-oxide gave a quasi-molecular ion at m/z 479 [Fig. 10(b)] in marked contrast to that of A_{7a}.

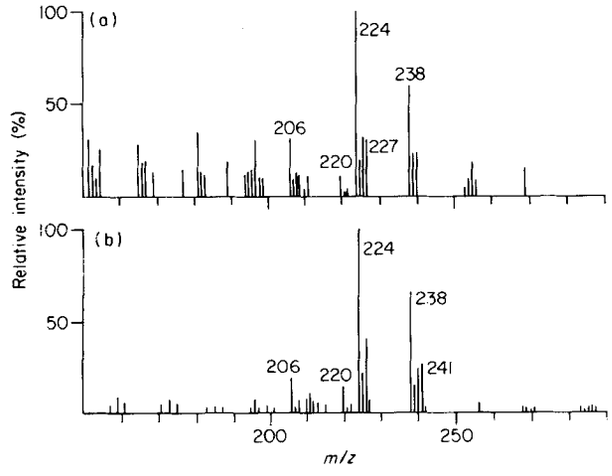


Figure 6. Mass spectra of (a) A_6 and (b) A_6 treated with diazomethane.

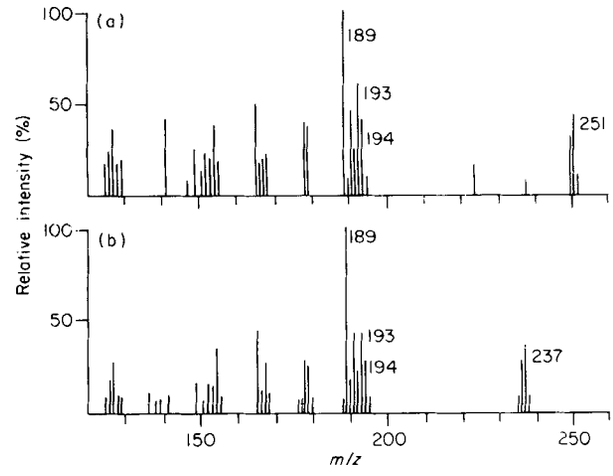


Figure 7. Mass spectra of (a) A_{7a} and (b) A_{7b}.

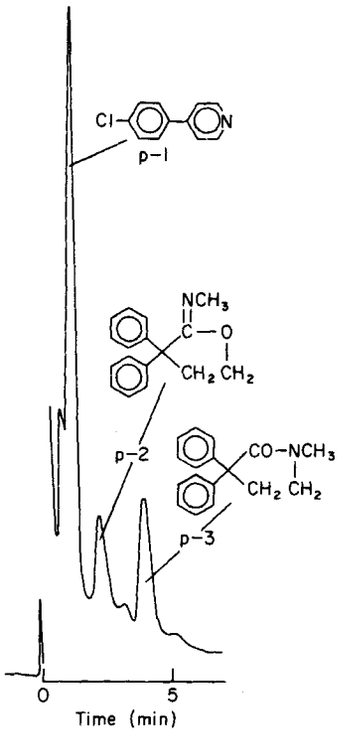
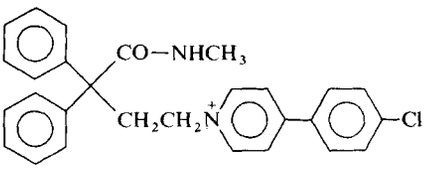


Figure 8. Total ion current chromatogram of A_{7a} without derivatization.

The above pyridinium structure is consistent with the EI mass spectrum of A_{7a} involving thermal degradation. This pyridinium derivative can be derived biologically



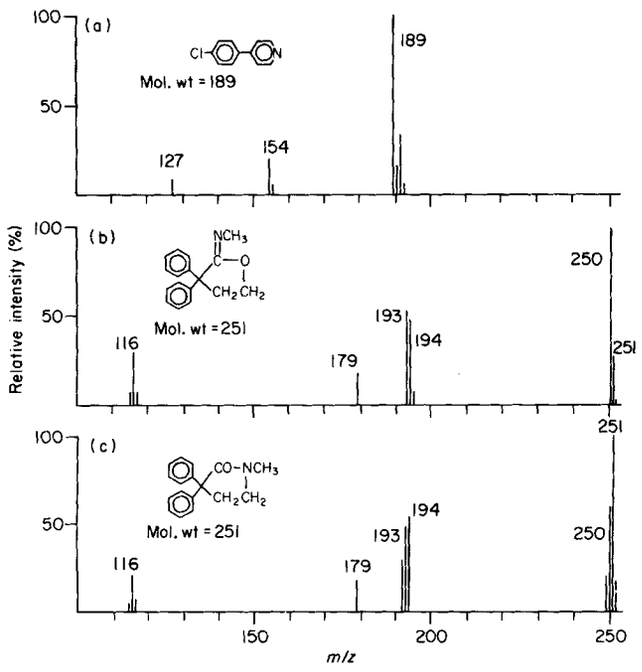


Figure 9. Mass spectra of (a) 4-(*p*-chlorophenyl) pyridine, (b) (tetrahydro-3,3-diphenyl-2-furylidene)methylamine and (c) 1-methyl-3,3-diphenyl-2-pyrrolidinone.

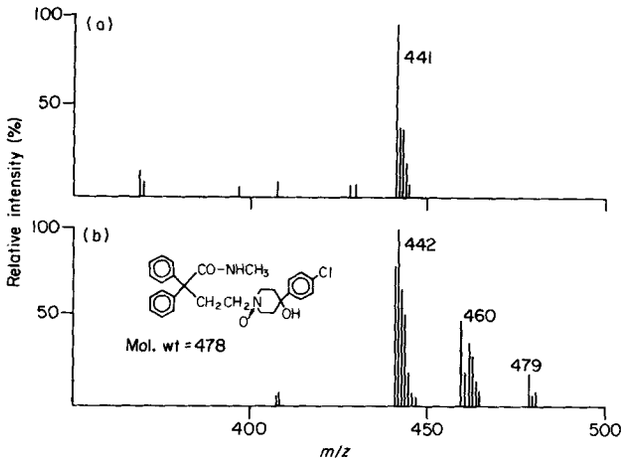
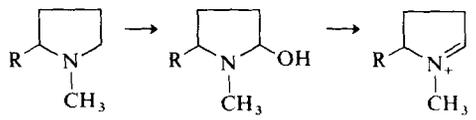


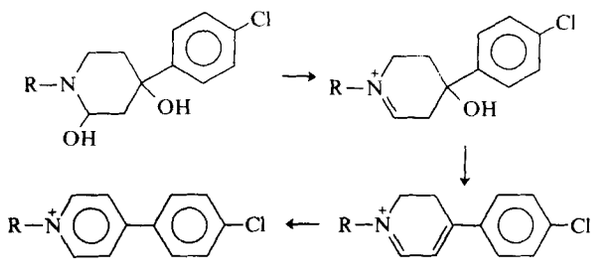
Figure 10. FD mass spectra of (a) A_{7a} (emitter current 17 mA) and (b) desmethylloperamide *N*-oxide (emitter current 15 mA).

via hydroxylation at the 2 or 3 position of the piperidine ring of desmethylloperamide. The 3-hydroxy group in the piperidine ring may be stable since 1-methyl-3,4-dihydroxy-4-phenylpiperidine exhibits a prominent molecular ion in the EI mass spectrum (Fig. 11). On the contrary, the 2-hydroxy group in the piperidine ring may be very unstable since in the metabolism of nicotine the product isolated was a metabolite of the dehydrated iminium ion, derived spontaneously from α-hydroxyl pyrrolidine.[9]



Scheme 2

Therefore, it is highly likely that A_{7a} was derived from a precursor, 2-hydroxyl desmethylloperamide, which underwent spontaneous conversion to the iminium, followed by dehydration of the 4-hydroxyl group and spontaneous aromatization.



Scheme 3

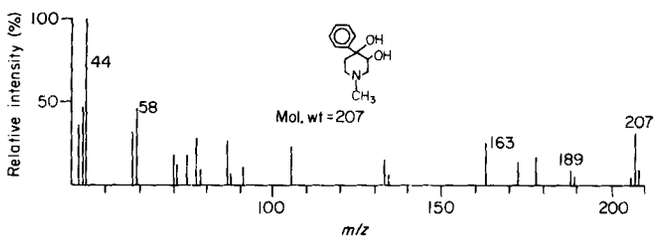
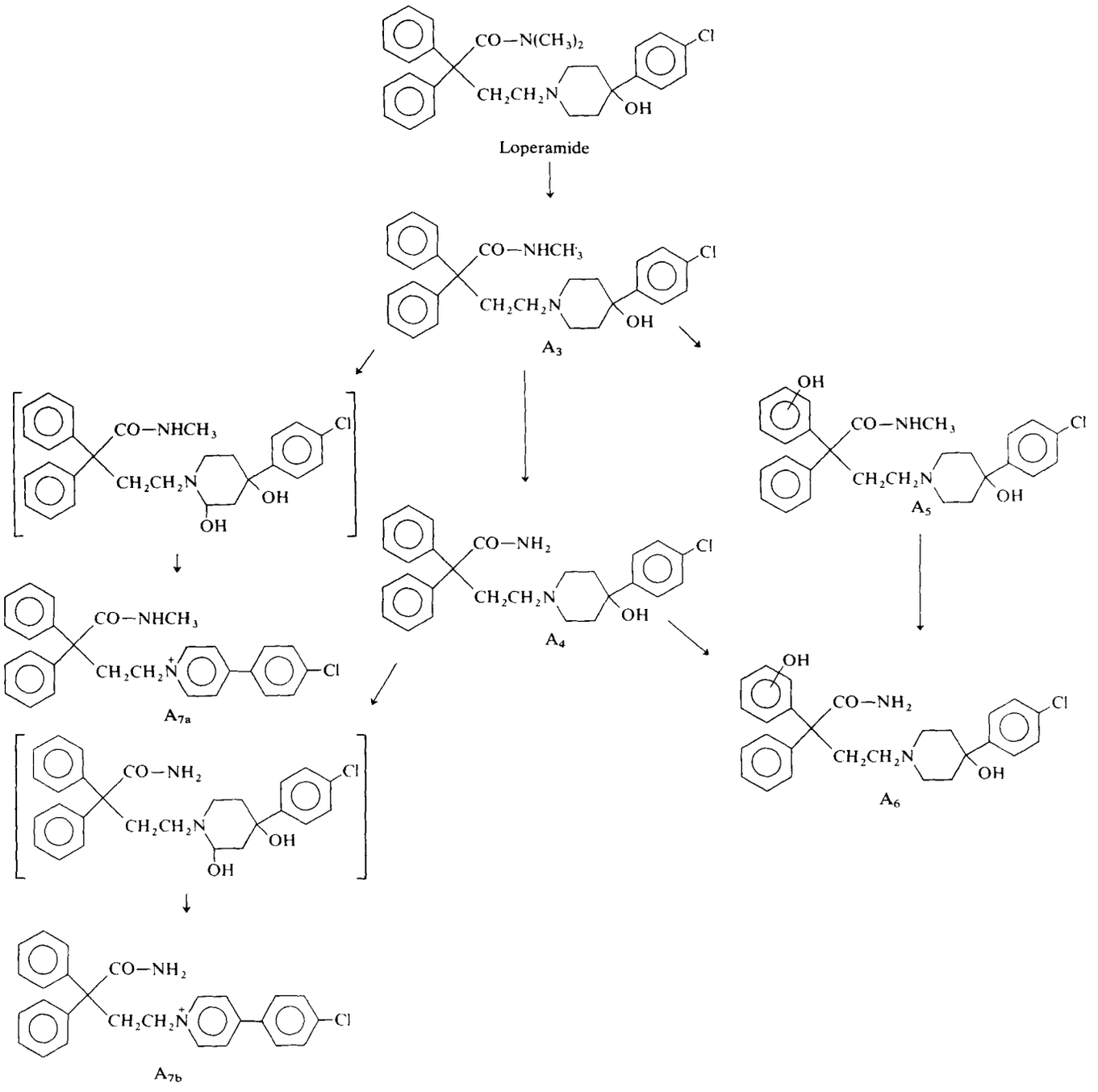


Figure 11. Mass spectrum of 1-methyl-3,4-dihydroxy-4-phenylpiperidine.



Scheme 4. Possible metabolic pathways of loperamide in rats.

The EI mass spectrum of A_{7b}, as described above, suggested that A_{7b} was a demethylated analogue of A_{7a}. A cluster at m/z 236–237 was due to 3,3-diphenyl-2-pyrollidinone and tetrahydro-3,3-diphenyl-2-furylideneamine, thermal degradation products in the direct probe, while a cluster at m/z 193–194 was associated with an α,α-diphenyl propenyl ion. Therefore, similarly to A_{7a}, A_{7b} was attributed to the pyridinium derivative of didesmethylloperamide derived from 2-hydroxyl didesmethylloperamide.

In urine, only two metabolites were isolated and identified to be desmethyl- and didesmethylloperamide, since [^{14}C]loperamide was excreted into urine in a small amount.

Metabolic pathway of loperamide

The dealkylation of loperamide proceeded predominantly in the rat prior to hydroxylation in the α-phenyl ring or in the piperidine ring, since desmethyl- or didesmethylloperamide was isolated, and not loperamide hydroxylated in either the α-phenyl ring or the piperidine ring.

Although 4-(p-chlorophenyl)-4-hydroxypiperidine was isolated in rat urine after administration of [^{3}H]loperamide labelled at the adjacent position to the chlorine substitution,[5] in the present study of [^{14}C]loperamide, corresponding acidic metabolite(s) seemed to be very minor as evident from the recovery of

radioactivity in the urinary acidic fraction. Therefore, oxidative *N*-dealkylation to give the substituted piperidine was, if any, a minor metabolic pathway for loperamide, in contrast to the major metabolic pathway for butyrophenones containing similar substituted piperidine moieties such as haloperidol.[10] Therefore, the metabolic pathway of loperamide in rat can be summarized as shown in Scheme 4. Further β-glucuronide conjugation of A_2–A_6 has been demonstrated in bile and urine in rats, which will be reported elsewhere.[8]

Acknowledgements

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