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## Note

# Characterization of the antidiarrhoeal loperamide by gas chromatography–mass spectrometry and application of the Hofmann degradation and Cope elimination reaction

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Loperamide, 4-(4-chlorophenyl)-4-hydroxy-N,N-dimethyl-$\alpha$,$\alpha$-diphenyl-1-piperidinebutyramide hydrochloride (R18 553) is a very potent, orally long-acting and specific antidiarrhoeal agent [1] The only analytical technique described for this drug is radioimmunoassay, which shows considerable cross-reactivity with the closely related compounds fluperamide and certain butyrophenone-type drugs [2] It was the aim of this study to establish a chromatographic method that would permit rapid qualitative analysis and identification of loperamide Being a tertiary amine, the drug should be amenable to the Hofmann degradation reaction, as described for butyrophenones, imipramine and certain phenothiazine derivatives [3,4] As an alternative, the Cope elimination reaction is discussed and the results are compared

## EXPERIMENTAL

### *Reagents*

Dimethylformamide (DMF) (silylation grade) was purchased from Pierce (Rockford, IL, U.S A ) Methyl iodide was obtained from Sigma (Munich,

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F R G.). Silver(I) oxide was supplied by Fluka (Buchs, Switzerland) Loperamide hydrochloride was a kind gift from Dr. V Nitsche (Biokinet, Vienna, Austria). All other reagents and solvents of analytical grade were purchased from Merck (Darmstadt, F.R.G.).

*The Hofmann degradation reaction [5]*

To the drug solution in 0 5 ml of benzene (free base form), 1 ml of methyl iodide was added and the mixture was left at room temperature for 30 min Solvent and reagent were removed under a stream of nitrogen at 50 °C, and the dry residue was redissolved in 1 ml of methanol. A knife-pinch of silver oxide was added along with 50 $\mu$l of water. The vial was shaken, and the silver oxide was removed by centrifugation. The clear supernatant was transferred to another vial and concentrated under a stream of nitrogen An aliquot of the solution was injected into the gas chromatographic–mass spectrometric (GC–MS) system Hofmann degradation of the hydroxide base of the quaternary ammonium compound occurred in the hot GC injector

*The Cope elimination reaction [6–8]*

A 100-$\mu$l of hydrogen peroxide (30% in water) was added to a solution of loperamide (free base) in 100 $\mu$l of methanol. The vial was shaken and left at room temperature for 15 h. Then 1 ml of methanol was added and the reaction mixture was dried in a vacuum centrifuge The residual aminoxide was dissolved in methanol, and an aliquot was injected into the gas chromatograph. Cleavage of the derivative occurred in the hot GC injector

*Gas chromatography–mass spectrometry*

A Finnigan 9610 gas chromatograph coupled to a Finnigan 4500 mass spectrometer was used The gas chromatograph was equipped with a DB-5 fused-silica capillary column (30 m$\times$0 25 mm I D , 0.25 $\mu$m film thickness) from J&W Scientific (Rancho Cordova, CA, U.S A.) The splitless Grob injector was kept at 300 °C. The column was kept at 100 °C for 1 min, then programmed to 300 °C at 25 °C/min and kept there until elution was complete The column was directly connected to the ion source of the mass spectrometer Helium was used as a carrier gas Electron impact (EI) spectra were recorded with an electron energy of 70 eV and an emission current of 0 2 A For chemical ionization (CI) methane was used as a reagent gas at an ion source pressure of $2.0 \cdot 10^{-5}$ Torr CI spectra were obtained at an electron energy of 120 eV and an emission current of 0 2 A.

## RESULTS AND DISCUSSION

*Hofmann degradation of loperamide*

The chromatogram obtained after the Hofmann elimination reaction of loperamide is given in Fig 1a. Peaks were identified by their EI and CI mass

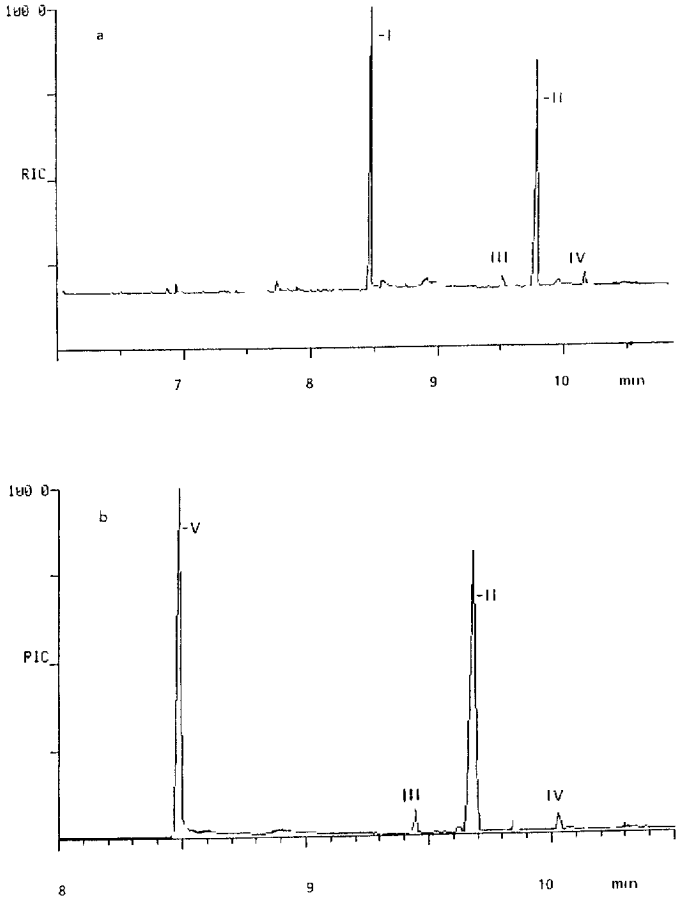



Fig 1 Partial ion chromatograms of loperamide (a) after Hofmann degradation of the quaternary ammonium hydroxide and (b) after Cope elimination of the aminoxide Peak numbers are defined in text

spectra, shown in Figs 2 and 3 Peaks I and II correspond to 4-(*p*-chlorophenyl)-4-hydroxy-N-methylpiperidine and $\alpha,\alpha$-diphenyl-3-en-N,N-dimethylbutyramide Apparently, bond fission during the Hofmann degradation reaction occurs predominantly at the non-cyclic alkyl residue of the quaternary ammonium compound (Fig. 4a) Besides that main degradation pathway, minute amounts of side-products are formed by loss of ethene (peak III) and addition of methanol (peak IV) to the olefinic fragment The latter side-reaction can be avoided by using dry DMF as the solvent for GC analysis

*Cope elimination of loperamide*

Fig. 1b shows the chromatogram obtained after Cope elimination reaction of loperamide Since Cope elimination of six-membered heterocycles does not

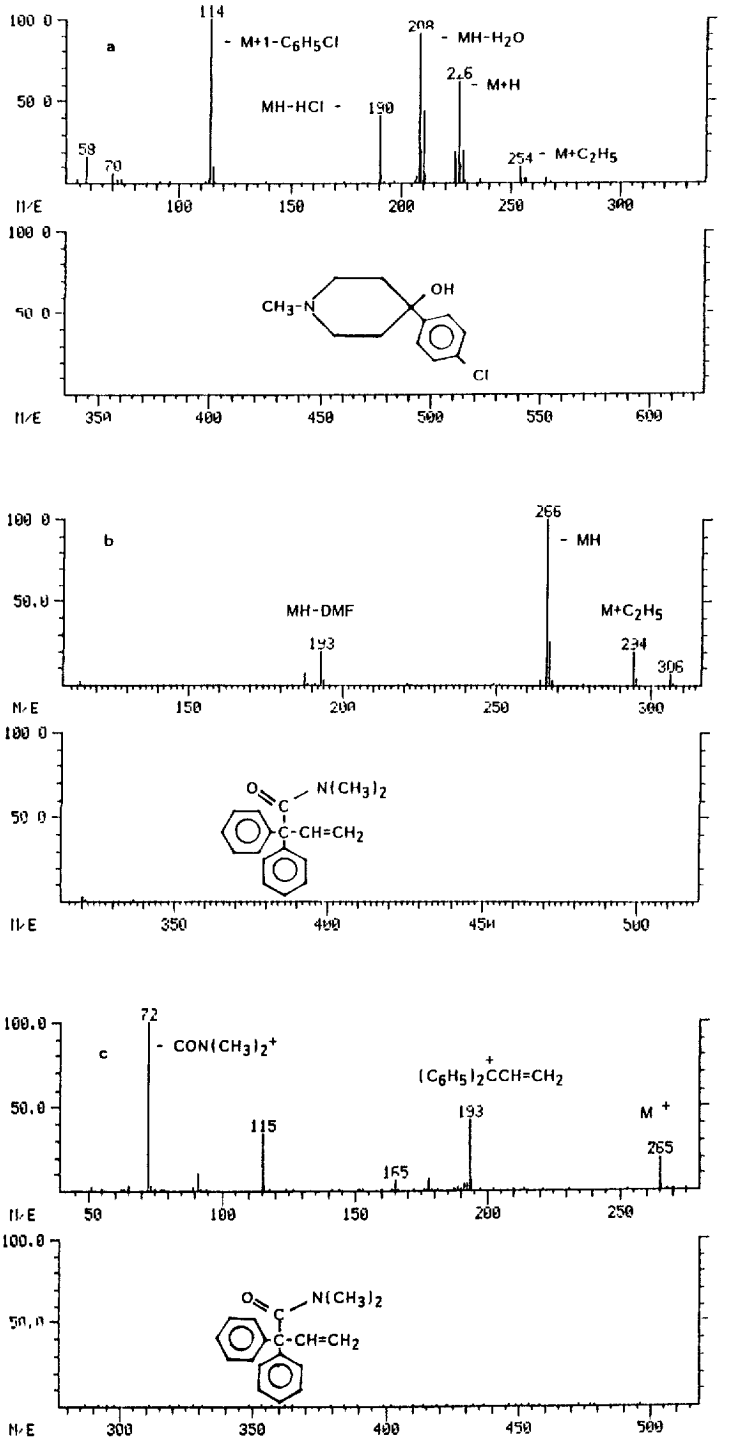



Fig 2 (a) CI mass spectrum of peak I (Fig 1), (b) CI mass spectrum of peak II (Fig 1), (c) EI mass spectrum of peak II (Fig 1)

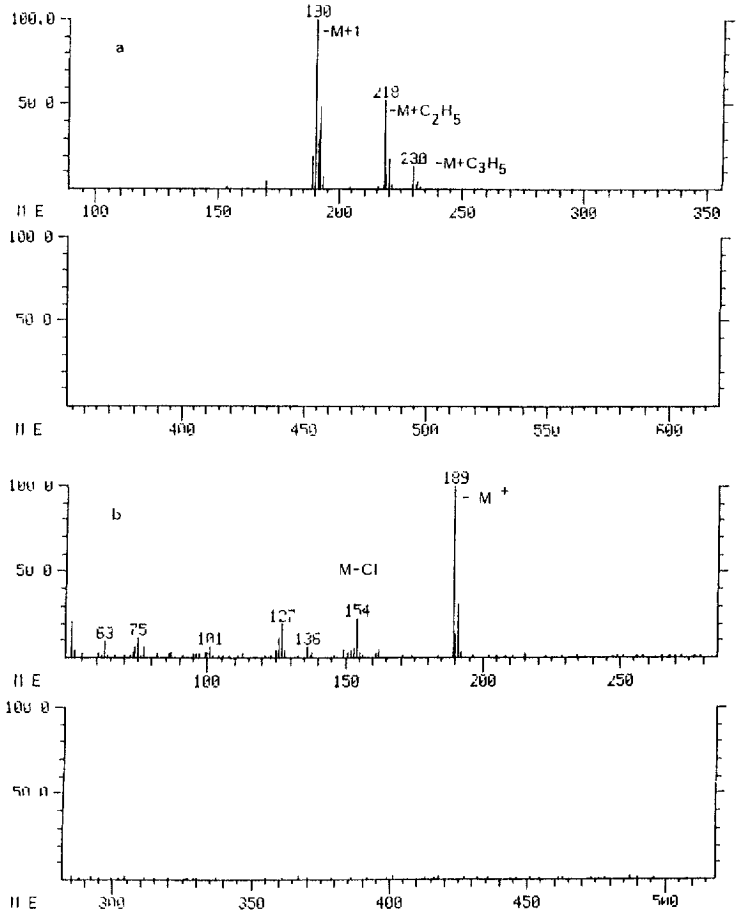
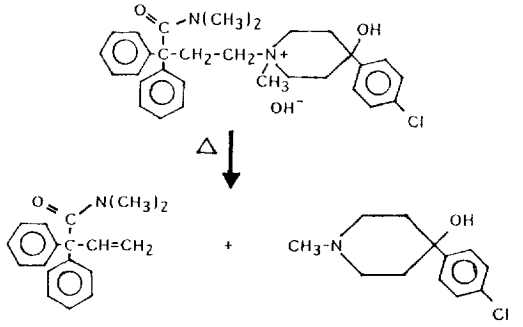



Fig 3 (a) CI and (b) EI mass spectra of peak V (Fig 1)

lead to ring opening but instead to elimination of non-cyclic alkyl chains [6], a similar chromatographic pattern as obtained under Hofmann degradation conditions was expected. EI- and CI-MS revealed that the olefinic fragment formed was identical with the one obtained after Hofmann degradation Additionally, the same side-products formed by ethene elimination and methanol addition were observed As indicated in Fig 4b, the second diagnostic fragment should correspond to 4-(*p*-chlorophenyl)-4-hydroxy-N-hydroxylpiperidine EI and CI mass spectra of peak V, however, showed a compound with a molecular mass of 189, being 38 mass units lower than the expected hydroxylamine (Fig. 3). A possible explanation would be thermal elimination of both hydroxyls as water and subsequent dehydrogenation to the corresponding substituted pyridine This hypothesis is supported by the fact that coinjection of the aminox-

a Hofmann degradation pathway of loperamide




b Cope elimination pathway of loperamide

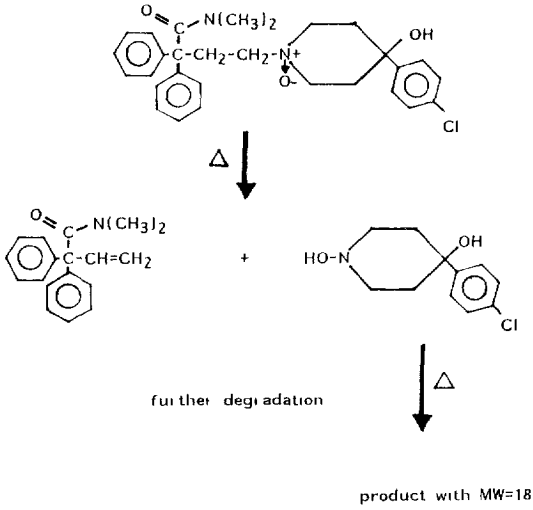



Fig 4 (a) Hofmann degradation pathway of the methylated quaternary ammonium hydroxide of loperamide (b) Cope elimination pathway of the aminoxide of loperamide

ide with N,O-bis(trimethylsilyl)trifluoroacetamide in pyridine leaves the intensity of the olefinic fragment unchanged, but leads to complete absence of compound V. Additionally, the EI mass spectrum (Fig 3b) shows a peak at *m/z* 127, which might arise from neutral loss (HCN) after elimination of chlorine, a fragmentation reaction frequently observed with aromatic heterocycles

The method described permits characterization of the antidiarrhoeal loperamide by both Hofmann degradation and Cope elimination. It is the first chromatographic assay described for the drug By monitoring the characteristic

masses of the fission products in the multiple ion detection mode, 50 ng of the compound can be detected by GC–MS under the conditions of positive ion chemical ionization Though detection limits are not sufficient to monitor therapeutic plasma levels of loperamide (which are usually below 1 ng/ml), the method should be useful in toxicological analysis and quality control of pharmaceuticals In addition, utilization of the Cope elimination reaction as an alternative to the Hofmann degradation reaction in microscale analysis of tertiary amines deserves further attention.

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