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## Note

# Identification of a metabolite of propafenone in human urine by means of high-performance liquid chromatography and gas chromatography—mass spectrometry

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Propafenone, 2′-(3-propylamino-2-hydroxy-propoxy)-3-phenyl-propiophenone, is a new antiarrhythmic drug which has proved effective in recurrent supraventricular and ventricular tachycardias, tachyarrhythmias and ectopic beats [1, 2].

The pharmacokinetic behaviour of the drug in man after intravenous and oral administration has been reported [3]. Propafenone undergoes extensive biotransformation [4] but the metabolism has not been elucidated.

Different methods for the determination of propafenone in plasma have been published [5—7]. It has also been suggested [6] that an unidentified peak eluting near propafenone in plasma extracts could be due to a metabolite. The purpose of the present study is to investigate the nature of this metabolite.

## EXPERIMENTAL

Propafenone·HCl was obtained from Knoll (Ludwigshafen, F.R.G.). *n*-Heptane, methylene chloride and absolute ethanol, HPLC grade, were purchased from Merck (Darmstadt, F.R.G.). Trifluoroacetic anhydride (TFAA) was obtained from Pierce (Rockford, IL, U.S.A.). All other solvents and chemicals were analytical grade.

### *High-performance liquid chromatography (HPLC)*

A Perkin-Elmer Series 3B liquid chromatograph equipped with a Merck

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LiChrosorb® Si 60 5-μm column (25 × 0.46 cm) was used. The mobile phase was dichloromethane—heptane—ethanol—water—ammonium hydroxide (180:120:200:20:0.2) at a flow-rate of 2.5 ml/min. The effluent was monitored with a Perkin-Elmer LC-75 spectrophotometer at 254 nm.

*Gas—liquid chromatography (GLC)*

A Perkin-Elmer Sigma 4 gas chromatograph equipped with a $^{63}$Ni electron-capture detector was employed. A 180 × 0.2 cm glass column packed with OV-101 3% on Gas-Chrom Q (100—120 mesh) was used. The instrumentation and the conditions were the same as reported previously for gas chromatographic analysis [7].

*Gas chromatography—mass spectrometry (GC—MS)*

GC—MS analysis was carried out on a Hewlett-Packard HP5792/5970A. A cross-linked fused-silica capillary column (12.5 m × 0.2 mm) coated with OV-1 as stationary phase was used. The carrier gas was helium at a flow-rate of 1.2 ml/min. The oven temperature was programmed from 70°C to 270°C at a rate of 12°C/min. Electron-impact spectra were recorded using 70-eV electron energy and a source temperature of 220°C.

*Extraction procedure and derivatization*

Plasma and urine samples from the same patient were extracted with benzene after alkalinization as described by Brode et al. [5]. Then 100 ml of urine were extracted using the same procedure to obtain a sufficient quantity of the metabolite for the GLC—electron-capture detection (ECD) and GC—MS analyses. The combined extracts were dried under a stream of nitrogen. The residue was reconstituted with methanol and injected into the liquid chromatograph.

The fractions corresponding to the metabolite were collected in a glass-stoppered tube and dried under a stream of nitrogen at 37°C. The residue was derivatized with TFAA in anhydrous toluene at 37°C for 1 h. Excess anhydride was removed by evaporation. The residue was reconstituted with cyclohexane and divided into two parts: the first was injected into the GLC—ECD system, the second into the GC—MS system.

## RESULTS AND DISCUSSION

It has been reported that less than 1% of unchanged propafenone is excreted in urine after its oral administration [4]. These findings suggest that the drug undergoes extensive metabolization and it has not been excluded that one or more metabolites contribute to the antiarrhythmic action of propafenone.

It is well known that the β-blocking agent propranolol, which has common structural features with propafenone, is metabolized to 4-hydroxy-propranolol and that this compound is pharmacologically active. It is therefore important to investigate the structures of the metabolites of propafenone.

Several authors [5, 6] have pointed out that one or two peaks, probably due to biotransformation, can occur in plasma extracts from patients who have taken propafenone orally. Our previous experience using HPLC [5] for

the determination of propafenone confirmed the existence of one peak eluting close to propafenone and with the same retention time in plasma and urine samples extracted by the same analytical procedure (Fig. 1). It is therefore reasonable that it is the same unidentified compound in both plasma and urine.

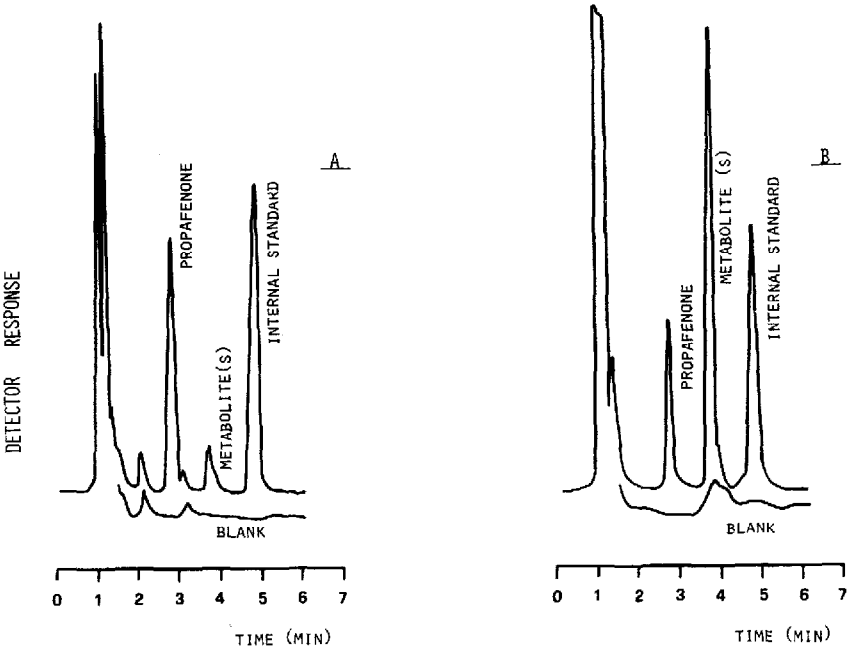


**Fig. 1. Representative HPLC chromatograms of plasma (1 ml, A) and urine (2 ml, B) samples after chronic administration of propafenone.**

Since the plasma concentration of the unknown compound is unsuitable for identification purposes, the corresponding fraction of urine was collected and analyzed. To obtain some preliminary information on the metabolite, the fraction was dried under a stream of nitrogen, derivatized with TFAA as described above and one portion injected into the GC—ECD system. The chromatogram in Fig. 2 shows two peaks eluting after propafenone: the first at 8.69 min, the second, much smaller than the first, at 10.03 min. These results indicate that the metabolite contains groups reacting with TFAA with a molecular weight probably higher than that of the propafenone derivative.

The second portion was injected into the GC—MS system under the conditions described above. Fig. 3 shows the total ion monitoring and the *m/e* 308 selective ion monitoring plots of the injected fraction.

Fig. 4 shows the mass spectrum of propafenone—TFA used as a reference for comparison with the spectrum of the unknown metabolite. Propafenone has an alkylaminohydroxypropoxy side-chain in common with most of the β-adrenoceptor antagonists; therefore its spectrum shows many fragment ions characteristic of the spectra of TFA β-blockers. These fragment ions are at *m/e* 308, 266, and 43 with high relative abundance, and at *m/e* 194, 168, 152, and 126 with low relative abundance. The attribution of these fragment

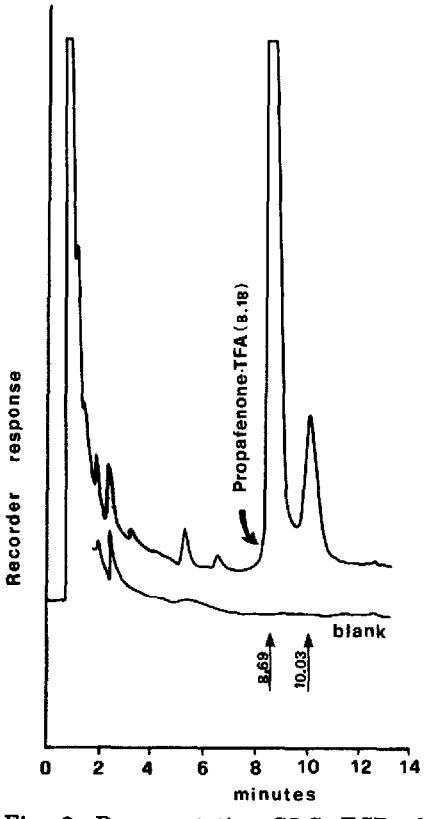


Fig. 2. Representative GLC—ECD chromatogram of the metabolite fraction from urine after derivatization with TFAA.

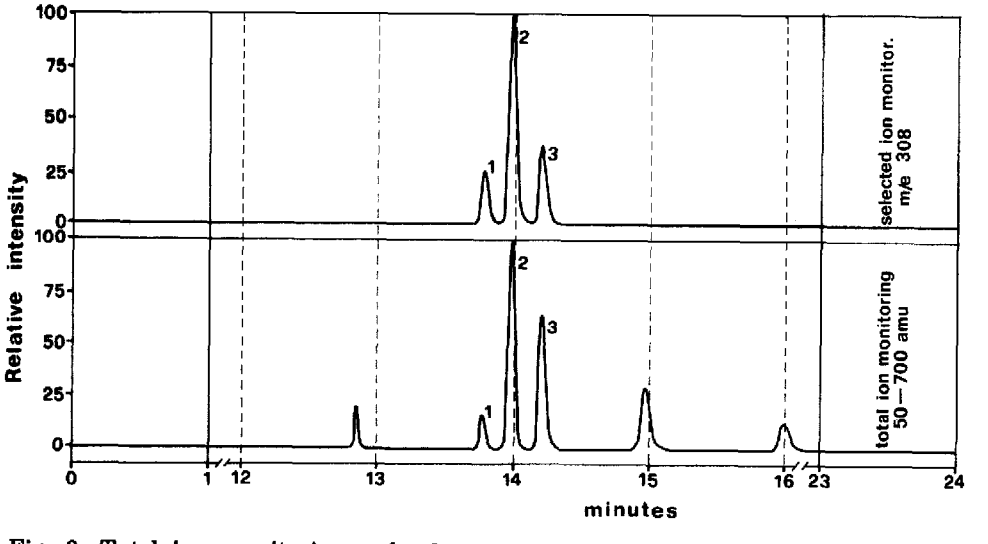


Fig. 3. Total ion monitoring and selective ion monitoring (*m/e* 308) of the metabolite fraction from urine after derivatization with TFAA. Peaks: 1 = propafenone•TFA, 2 = hydroxylated propafenone•TFA and 3 = unidentified compound.

ions and their relative fragmentation mechanism is described in the literature [8]. The propafenone—TFA molecular ion at *m/e* 533 with low relative abundance is consistent with formation of a di-(trifluoroacetyl) derivative.

The fragment ion at *m/e* 225 could be reasonably ascribed to the remaining part of the molecule after the loss of the alkylaminohydroxypropoxy side-chain. Its intensity should be relatively weak because, after fragmentation, the charged ion is *m/e* 308. The fact that the relative abundance of *m/e* 225 compared with that of *m/e* 308 is 6.8% supports our hypothesis.

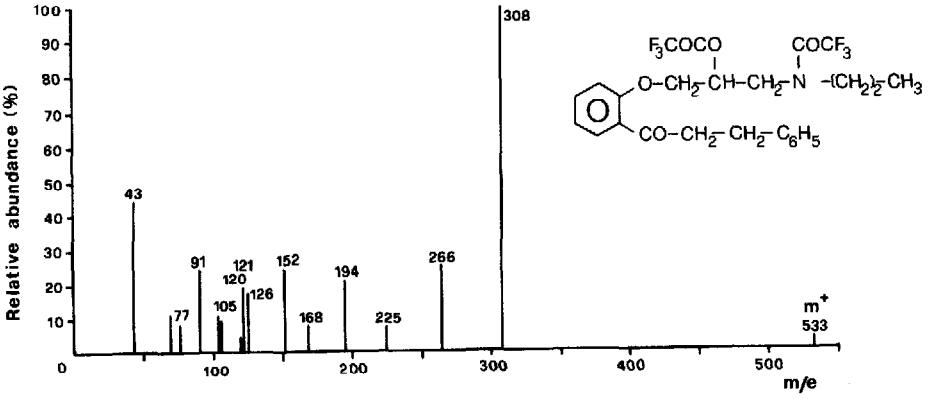


Fig. 4. Mass spectrum of propafenone—TFA and its structural formula.

The fragment ions at *m/e* 77, 91 and 105 can be explained on the basis of the literature on alkylbenzenic compounds [9]. In analogy with the fragmentation behaviour of alkylbenzenic ethers and phenylalkylketones [9], the fragment ions at *m/e* 120 and 121 were ascribed to the structures shown below.


*m/e* 120 *m/e* 121

The mass spectra of the three peaks shown in Fig. 3 were recorded. The mass spectrum of the first peak was found to be identical to that of propafenone—TFA. This could be explained by a propafenone contamination during the collection of the HPLC fraction.

The mass spectrum of second peak is shown in Fig. 5. The molecular ion of this compound is at *m/e* 645, i.e. 112 mass units higher than propafenone—TFA. The same difference was found between the ions at *m/e* 232 and 233 of this compound and ions at *m/e* 121 and 120 of propafenone—TFA, and could reasonably be ascribed to a $-OCOCF_3$ group. This hypothesis is consistent with the fact that hydroxylation of the aromatic ring is a metabolic pathway common to these compounds [10]. The presence also of the fragment ions at *m/e* 77, 91 and 105 in the spectrum of the metabolite indicates that the hydroxylation occurs at the two-fold substituted aromatic ring. Moreover, the

possibility of metabolic degradation of the alkylaminohydroxypropoxy side-chain can be excluded because all the characteristic fragment ions are present in the spectrum of the metabolite.

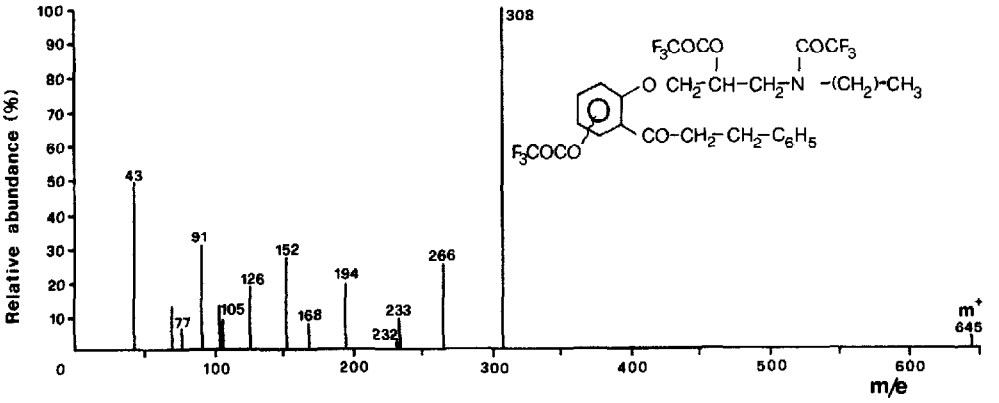


Fig. 5. Mass spectrum of the second peak corresponding to the metabolite.

The mass spectrum of the third peak was very difficult to interpret on the basis of the common metabolic pathways; the structure of this substance thus remains an open problem.

These findings indicate that propafenone undergoes metabolic biotransformation and that the main metabolite is a hydroxylated derivative.

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