Journal of Chromatography, 145 (1978) 413—420
Biomedical Applications

CHROMBIO. 148

STUDIES ON THE METABOLISM OF 2,4′-ISOBUTYLPHENYLPROPIONIC ACID (IBUPROFEN) BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

DIALYSIS FLUID, A CONVENIENT MEDIUM FOR STUDIES ON DRUG METABOLISM

JON ELLING PETTERSEN*, GUNNAR ARNFINN ULSAKER and EGIL JELLUM

Institute of Clinical Biochemistry, University of Oslo, Rikshospitalet, Oslo, and National Centre for Medicinal Products Control, Oslo (Norway)

(Received December 6th, 1977)

SUMMARY

2,4′-Isobutylphenylpropionic acid (ibuprofen) has previously been demonstrated to yield four urinary metabolites, formed by ω1-, ω2- and ω3-hydroxylation and by a further oxidation of the primary alcohol of the ω1-hydroxylated metabolite to a carboxyl group. By synthesis and gas chromatography—mass spectrometry the suggested structure of the ω3-hydroxylated metabolite was verified in the present study. Moreover, a new metabolite, 2,4′-carboxyphenylpropionic acid, was demonstrated to be present in substantial amounts in dialysis fluid from a nephrectomized patient. In such patients ingested drugs cannot be excreted in the urine, but are metabolized to end products. Thus, dialysis fluid may be a convenient medium for studies on drug metabolism.

INTRODUCTION

The drug ibuprofen (2,4′-isobutylphenylpropionic acid) is a non-steroidal agent with anti-inflammatory, analgesic and antipyretic properties, which has gained acceptance in the treatment of rheumatoid arthritis and other rheumatic conditions. Using e.g. combined gas chromatography—mass spectrometry (GC—MS), previous workers [1, 2] have detected four metabolites, viz. the metabolites 1—4 shown in Fig. 1. Metabolites 2 and 4 have been synthesized [1], whereas metabolites 1 and 3 have been identified by interpretation of mass spectra of appropriate derivatives of the metabolites [2]. There was some uncertainty, however, with regard to the interpretation of the mass spectrum of

*To whom correspondence should be addressed at the Institute of Clinical Biochemistry.

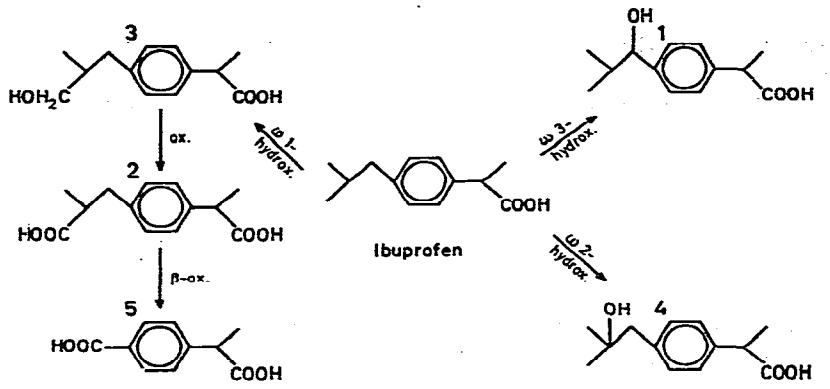

Fig. 1. Pathways of 2,4′-isobutylphenylpropionic acid (ibuprofen) metabolism and chemical structures of the demonstrated metabolites.

the postulated metabolite 1 [2].

The structure of metabolite 2, with the methyl branching in the longest side chain in the α-position to the carboxyl group, suggests that this compound may be further degraded by β-oxidation to yield 2,4′-carboxyphenylpropionic acid. This metabolite has not been demonstrated previously. A possible explanation might be that metabolite 2 is rapidly excreted in the urine and thus escapes a subsequent β-oxidation. In nephrectomized patients, however, drugs and their metabolites can not be excreted and may therefore be degraded to end products.

In the present study some possible metabolites of 2,4′-isobutylphenylpropionic acid were synthesized. Subsequently these metabolites were searched-for by combined GC—MS in dialysis fluid from a nephrectomized patient and in the urine from a healthy volunteer, both persons taking ibuprofen perorally. A new metabolite was identified (metabolite 5 in Fig. 1), the suggested structure of metabolite 1 [2] was verified, and it was found that dialysis fluid is a convenient medium for studies on drug metabolism. A preliminary report with some results from the present study has been presented previously [3].

MATERIALS AND METHODS

Chemicals

Ibuprofen was delivered by Boots (Nottingham, Great Britain). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce (Rockford, Ill., U.S.A.). N-Nitrosomethylurea for diazomethane production was delivered by K and K Labs. (Calif., U.S.A.). Stationary phases for gas chromatography (OV-17 and SE-30) and solid support (Gas-Chrom Q) were obtained from Applied Science Labs. (State College, Pa., U.S.A.). 2,4′-Carboxyphenylpropionic acid, 2,4′-(1-hydroxy-2-methylpropyl) phenylpropionic acid, 2,4′-(1-oxo-2-methylpropyl)phenylpropionic acid, and 3-hydroxy-2,4′-isobutylphenylpropionic acid were synthesized [4].

Dialysis fluid and urine samples

Dialysis fluid was obtained from a 24 year-old nephrectomized female under-

going hemodialysis regularly, twice a week. She suffered from rheumatoid arthritis and was daily given perorally two 200-mg tablets of ibuprofen. Special precautions were taken for collection of dialysis fluid. The flow of fresh dialysis fluid was diminished to obtain near-equilibrium passage of various metabolites across the dialysis membrane. The first 5 l of dialysis fluid were collected, divided into 40-ml portions and lyophilized. The samples were redissolved in 5 ml of water and subsequently treated exactly like the urine samples.

A 12-h urine sample was collected from a healthy, 34 year-old, male volunteer who had ingested three 200-mg tablets of ibuprofen. Five ml aliquots of the sample were used for the further studies.

Extraction of organic acids from dialysis fluid or urine samples was performed with diethyl ether (3 × 3 volumes) after adjusting the pH to 1 with 6 *M* HCl. The combined extracts were dried over anhydrous sodium sulphate.

Methylation was performed with diazomethane liberated from N-nitrosomethylurea. Ethylation was performed with ethanol—HCl. Trimethylsilylation was carried out in pyridine with BSTFA.

Gas chromatography and mass spectrometry

Two combined GC—MS instruments were used. One instrument consisted of a Varian 1440 gas chromatograph, a molecular separator of the glass frit type (kept at 230°) and a single-focusing mass spectrometer, type Varian CH 7 (Varian-MAT, Bremen, G.F.R.), operated with an ionisation energy of 70 eV. The gas chromatograph was equipped with a packed column (2 m × 1/4 in. O.D.) filled with 10% OV-17 on Gas-Chrom Q, 80—100 mesh. Helium was used as carrier gas (30 ml/min).

Multiple ion detection (selected ion monitoring, mass fragmentography) was carried out using a Varian 112 mass spectrometer fitted with a glass capillary column (SE-30, 25 m × 0.25 mm; LKB, Stockholm, Sweden) connected directly to the ion source.

Both GC—MS instruments were connected on-line to a computer system (Spectro System 100 MS; Varian-MAT). For the calibration of the mass spectrometers perfluorokerosene was used as reference substance.

High resolution mass spectrometric analyses were undertaken in an AEI MS 902 double-focusing instrument (70 eV ionizing energy, 100 μA ionization current). The samples were introduced by the heated direct-inlet probe. Perfluorotributhylamine was used as reference substance.

RESULTS

Fig. 2 shows gas chromatograms of the methylated diethyl ether extract of acidified dialysis fluid from a nephrectomized patient (a) and of urine from a healthy volunteer (b), both persons ingesting ibuprofen. In the urine sample large amounts of unmetabolized ibuprofen were found, together with only small amounts of metabolite 5. In the dialysis fluid, however, no trace of the unmetabolized drug could be detected, whereas substantial amounts of one of the metabolic end products of the drug, metabolite 5, were found. In addition to the drug metabolites several well-known metabolites normally occurring in the urine, e.g. hippuric acid, were observed in the chromatogram [5].

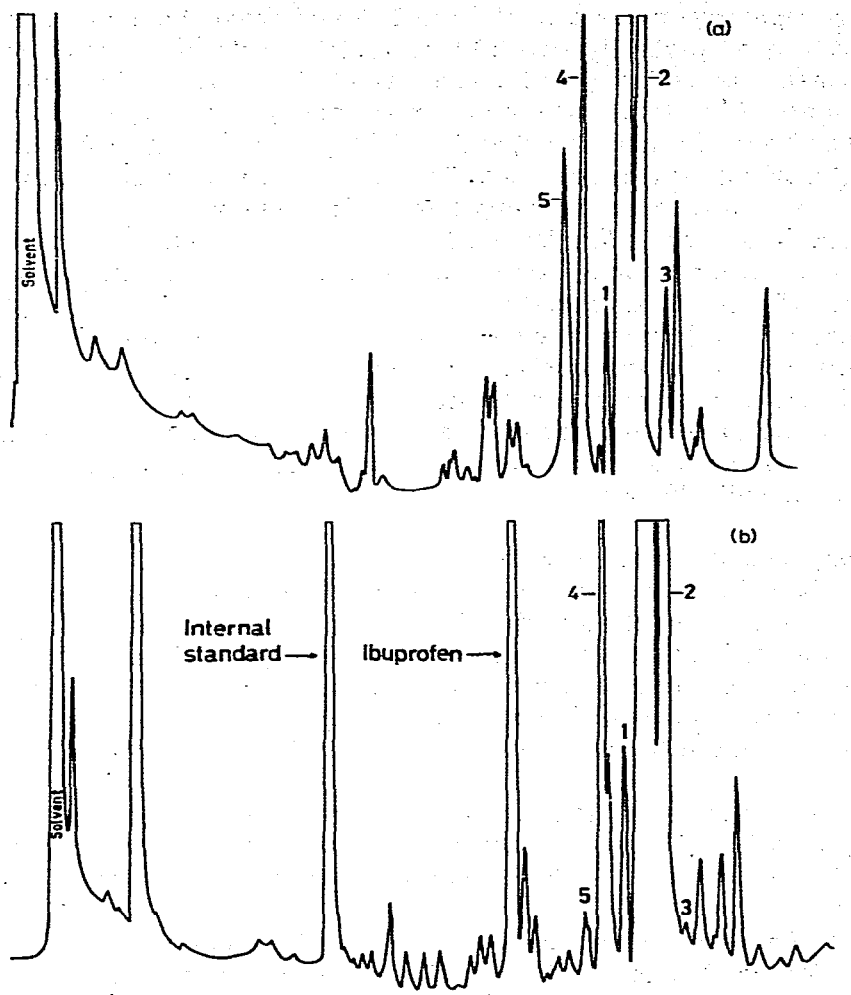

Fig. 2. Gas chromatograms of methylated diethyl ether extracts of acidified dialysis fluid from a nephrectomized patient (a) and urine from a healthy volunteer (b), both persons having ingested ibuprofen. GC was performed in a packed column (10% OV-17 on Gas-Chrom Q, 80—100 mesh), the temperature was programmed 80—300° (8°/min), and carrier gas was helium (30 ml/min). The GC peaks have been identified as the following compounds (as methyl esters): 1=2,4′-(1-hydroxy-2-methylpropyl)phenylpropionic acid; 2=2,4′-(2-carboxypropyl)phenylpropionic acid; 3=2,4′-(2-hydroxymethylpropyl)phenylpropionic acid; 4=2,4′-(2-hydroxy-2-methylpropyl)phenylpropionic acid; 5=2,4′-carboxyphenylpropionic acid.

The metabolites giving rise to peaks 2 and 4 (metabolites 2 and 4 in Fig. 1) have been previously synthesized and identified as 2,4′-(2-carboxypropyl)-phenylpropionic acid and 2,4′-(2-hydroxy-2-methylpropyl)phenylpropionic acid [1]. The metabolites giving rise to peaks 1 and 3 (metabolites 1 and 3 in Fig. 1) have been detected and characterized by Brooks and Gilbert [2] using combined GC—MS. They identified the metabolites as 2,4′-(1-hydroxy-2-methylpropyl)phenylpropionic acid and 2,4′-(2-hydroxymethylpropyl)phenylpropionic acid.

In the present work the four mentioned metabolites have been studied both as methyl esters and as methyl ester—trimethylsilyl (TMS) ethers, and the findings of Brooks and Gilbert [2] have been confirmed. With regard to the interpretation of the mass spectrum of the methyl ester—TMS ether of metabolite 1 they stated: "The abundant ion at *m/z* 133 was at first ascribed to an impurity retaining the unsubstituted isobutylphenyl moiety, but was still formed from a purified sample and remain unassigned pending further examination". The postulated metabolite 1, i.e. 2,4′-(1-hydroxy-2-methylpropyl)phenylpropionic acid, was therefore synthesized [4]. Fig. 3 shows the mass spectrum of the

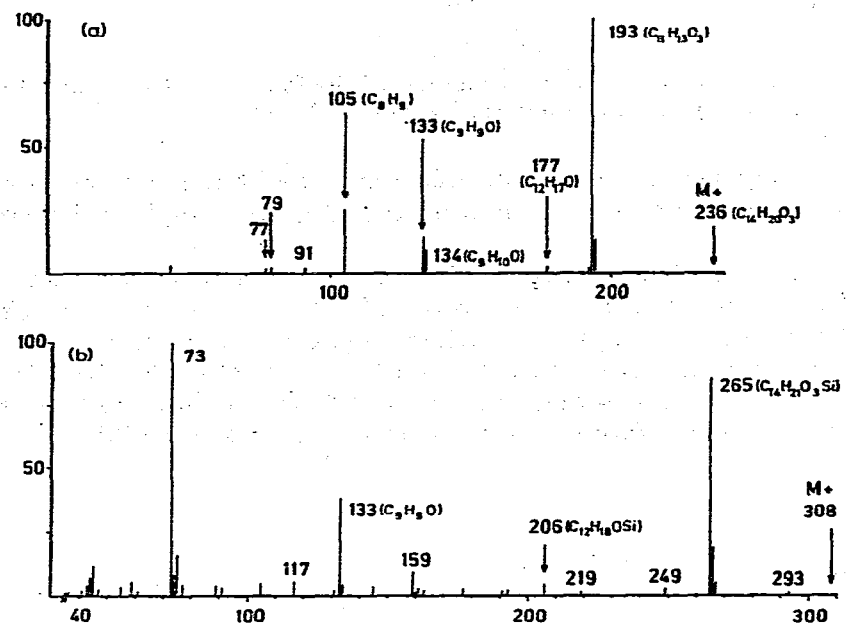

Fig. 3. Mass spectra of 2,4′-(1-hydroxy-2-methylpropyl)phenylpropionic acid as methyl ester (a) and as methyl ester—TMS ether (b). The empirical formulae (as indicated in the figure) have been determined by high-resolution mass spectrometry.

methyl ester of this compound (a) and the mass spectrum of the methyl ester—TMS ether (b). The empirical formulae (as indicated in the figure) of the various fragments have been determined by high resolution mass spectrometry. A suggested scheme for the formation of the ion at *m/z* 133 in the mass spectrum from the methyl ester—TMS ether of 2,4′-(1-hydroxy-2-methylpropyl)phenylpropionic acid is shown in Fig. 4.

The synthesized 2,4′-(1-hydroxy-2-methylpropyl)phenylpropionic acid had an identical mass spectrum as well as identical GC retention time with metabolite 1, both as methyl ester and as methyl ester—TMS ether.

Two other possible metabolites from 2,4′-isobutylphenylpropionic acid were synthesized, viz. 2,4′-(1-oxo-2-methylpropyl)phenylpropionic acid and 3-hydroxy-2,4′-isobutylphenylpropionic acid. The latter might be formed by an ordinary ω1-hydroxylation. Both of these compounds were searched for, first

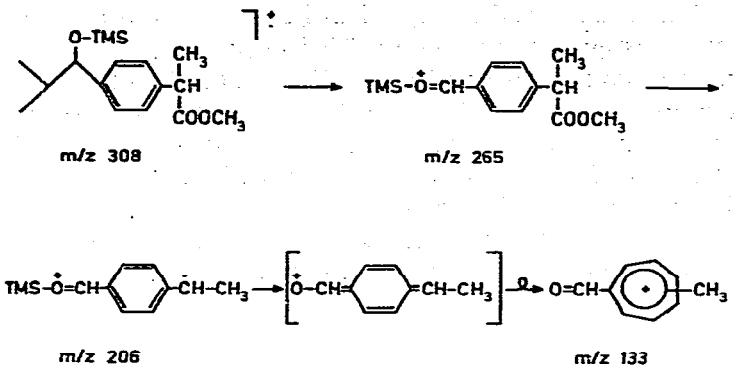

Fig. 4. Suggested fragmentation scheme for the formation of the ion at *m/z* 133 from 2,4'-(1-hydroxy-2-methylpropyl)phenylpropionic acid methyl ester—TMS ether.

by mass chromatography and later by multiple ion detection. However, not even traces of these compounds could be demonstrated in the samples (dialysis fluid or urine).

Peak 5 (Fig. 2) was identified as follows. The mass spectrum of this peak is shown in Fig. 5 (b). The molecular ion is at *m/z* 222; the fragment ions at *m/z* 191 and *m/z* 163 indicate a methyl ester. When the sample was treated with ethanol—HCl, the retention time of the peak was slightly increased and in the new mass spectrum (not shown) the molecular ion was found at *m/z* 250, thus showing the presence of two carboxylic groups. Ions at *m/z* 205 and *m/z* 177

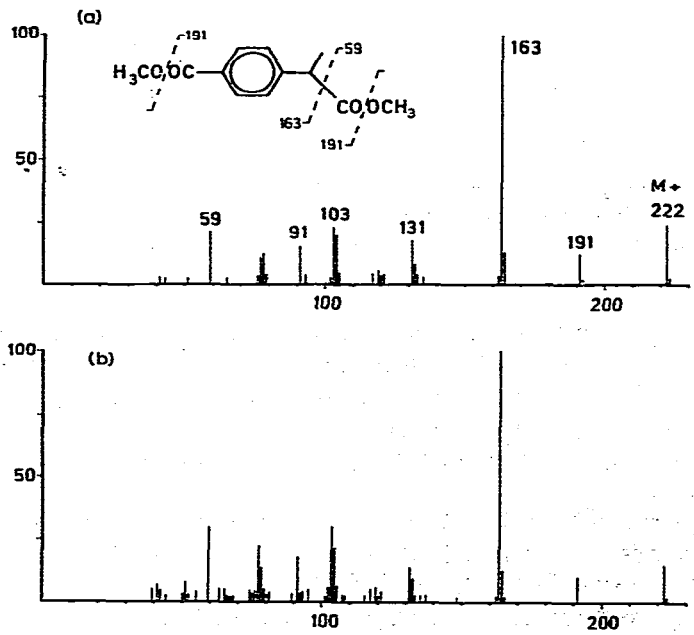

Fig. 5. Mass spectrum of authentic 2,4'-carboxyphenylpropionic acid dimethyl ester (a) and of the methylated ibuprofen metabolite (peak 5 in Fig. 2) detected in dialysis fluid from a nephrectomized patient (b).

demonstrated loss of an ethoxy and a carboethoxy group. The absence of hydroxyl groups was confirmed by the unchanged retention time and mass spectrum after trimethylsilylation of the dimethyl ester. The postulated structure of the new metabolite is shown in Fig. 1 (metabolite 5). This compound, viz. 2,4′-carboxyphenylpropionic acid, was synthesized [4], and the mass spectrum of the dimethyl ester is shown in Fig. 5 (a). This mass spectrum is identical to the mass spectrum of the methylated metabolite 5. Moreover, the two compounds had identical gas chromatographic retention times (0.50 relative to 2,4′-(2-carboxypropyl)phenylpropionic acid dimethyl ester in an OV-17 column, isothermally at 205°).

In the 2-methylpropionyl side chain of metabolite 2 (Fig. 1) the methyl branching is in the α-position to the carboxyl group. Thus, it is most likely that the new metabolite, 2,4′-carboxyphenylpropionic acid, is formed from metabolite 2 by an ordinary β-oxidation.

DISCUSSION

It has been shown by others [1, 2] and by us [3] that the metabolism of 2,4′-isobutylphenylpropionic acid takes place in the isobutyl side chain. Derivatives are formed by ω1-, ω2- and ω3-hydrolation and by a further oxidation of the ω1-hydroxylated metabolite. This is in accordance with the findings of Ruelius et al. [6] concerning the metabolism of the hypoglycemic agent 2-p-methoxybenzenesulphonamido-5-isobutyl-1,3,4-thiadiazole and our own findings concerning the metabolism of 4-isobutylphenylacetic acid [7].

Another possible site of attack for the ω-hydroxylation system would be at the 3-position in the propionic acid part of 2,4′-isobutylphenylpropionic acid. In the present study, however, not even traces of this postulated metabolite could be detected. Thus, it seems as if the neighbouring carboxylic group protects this methyl group from the ω1-hydroxylating enzymes.

The new metabolite, 2,4′-carboxyphenylpropionic acid (metabolite 5, Fig. 1), may be formed from 2,4′-(2-carboxypropyl)phenylpropionic acid (metabolite 2, Fig. 1) by β-oxidation. The reason why the new metabolite has not been previously detected may be that its precursor is rapidly excreted in the urine and thus for a large part escapes β-oxidation. In the nephrectomized patient in the present study the ingested drug could not be excreted in the urine, but was metabolized to end products. Such a product, 2,4′-Carboxyphenylpropionic acid, was found in substantial amounts in the dialysis fluid sample from the nephrectomized patient, whereas only small amounts were found in the urine sample from the healthy volunteer. Thus, we suggest that analyses of dialysis fluid from nephrectomized patients who for clinical reasons have to be given a certain drug, may yield new information on the metabolism of that particular drug.

ACKNOWLEDGEMENTS

The technical assistance of Miss Live Horn and Mr. Per Helland is gratefully acknowledged. Through the kind co-operation of the Chemistry Department

(Mr. G. Hvistendahl), University of Oslo, we had access to the high-resolution mass spectrometer.

REFERENCES

1 S.S. Adams, E.E. Cliffe, B. Lessel and J.S. Nicholson, J. Pharm. Sci., 56 (1967) 1686.
2 C.J.W. Brooks and M.T. Gilbert, J. Chromatogr., 99 (1974) 541.
3 J.E. Pettersen, B. Blessington and E. Jellum, Trans. 15th Meet. Scand. Soc. Clin. Chem. Clin. Physiol., Aarhus, Denmark 1975, p. 160.
4 G.A. Ulsaker and J.E. Pettersen, in preparation.
5 T.L. Masimore, H. Veening, W.J.A. VandenHeuvel and D.A. Dayton, J. Chromatogr., 143 (1977) 247.
6 H.W. Ruelius, D.C. de Jongh and S.R. Shrader, Arzneim.-Forsch., 20 (1970) 115.
7 J.E. Pettersen, Biomed. Mass. Spectrom., in press.