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STUDIES OF URINARY METABOLITES OF 2-(4-ISOBUTYLPHENYL)PROPIONIC ACID BY GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Human urinary metabolites of the oral anti-rheumatic drug RS-2-(4-isobutylphenyl)propionic acid ("ibuprofen") have been further characterised by gas chromatography–mass spectrometry of appropriate derivatives. Gas-phase analytical resolution of diastereomeric amides formed with R-(+)-α-phenylethylamine showed that the excreted drug was enriched in the (+)-enantiomer. Four other metabolites resulted from oxidative transformations of the isobutyl group; the structures of two of these had previously been reported by other workers.

Retention index values (for OV-1, OV-17 and QF-1 phases) are recorded, together with salient mass spectrometric data.

INTRODUCTION

The drug "ibuprofen", RS-2-(4-isobutylphenyl)propionic acid, is a powerful anti-rheumatic agent that has gained wide acceptance in the treatment of rheumatoid arthritis[1,2]. The racemic character of the drug, together with the comparatively high therapeutic dosage (typically 0.6–1.2 g/day) prompted our interest in the stereochemical aspects of its metabolism, and in the possible formation of biologically active metabolites. Previous workers[3,4] have detected four metabolites, of which two were identified as (+)-2,4′-(2-hydroxy-2-methylpropyl)phenylpropionic acid and (+)-2,4′-(2-carboxypropyl)phenylpropionic acid. These results therefore indicated some stereoselectivity in the metabolic oxidation of ibuprofen.

We now report a study of urinary metabolites isolated after the oral administration of ibuprofen. The enantiomeric composition of the excreted drug has been determined by gas–liquid chromatographic (GLC) analysis of derived diastereomeric amides[5]. Structures of other metabolites have been assigned by gas–liquid chromatography–mass spectrometry (GLC–MS) and in some cases also by NMR spectrometry.

EXPERIMENTAL

Materials and methods

"Brufen" (ibuprofen; The Boots Company, Nottingham, Great Britain) was

kindly supplied by Dr. G. G. Browning (Western Infirmary, Glasgow, Great Britain). R-(+)-α-Phenylethylamine was obtained from Fluka (Buchs, Switzerland); [d_{18}]-bis (trimethylsilyl)acetamide, from Merck Sharp & Dohme, Montreal, Canada; $LiAl^2H_4$, from Ciba (Basle, Switzerland); silica gel "for dry-column chromatography", from Woelm (Eschwege, G.F.R.). Preparative TLC was performed on glass plates coated with Kieselgel HF_{254} (layer thickness 0.5 mm). Bands were located with UV light.

GLC was carried out using a Pye Series 104 chromatograph and a Perkin-Elmer 881 chromatograph, both with flame ionisation detectors. Nitrogen was used as carrier gas. "Silanised" glass columns of 3–4 mm I.D. and 1.5–5 m length were packed with 1% SE-30, 1% OV-1, 1% OV-17, or 1% QF-1 coated on Gas-Chrom Q (100–120 mesh; Applied Science Labs., State College, Pa., U.S.A.). The 5-m columns (SE-30, OV-17) were used for the analytical resolution of diastereomers. GLC was carried out at the temperatures indicated in the tables.

GLC–MS was effected, with helium as carrier gas, using an LKB 9000 instrument equipped with a 3-m glass column packed with 1% OV-1, at an electron energy of 70 eV, an ion source temperature of 270° and a separator temperature of 270°.

NMR spectra were recorded at 100 MHz on a Varian HA100 instrument, using $CDCl_3$ as solvent.

Preparation of derivatives

Methylation of acids was effected with diazomethane in diethyl ether.

Trimethylsilylation of alcohols was carried out in pyridine with hexamethyldisilazane–trimethylchlorosilane (5:1) at 60°. Perdeuterated trimethylsilyl ethers were formed with [d_{18}]-bis(trimethylsilyl)acetamide, under the same conditions.

Chloromethyldimethylsilyl (CMDMS) ethers were prepared with 1,3-bis-(chloromethyl)-1,1,3,3-tetramethyldisilazane–chloromethyldimethylchlorosilane (2:1) in pyridine for 1 h at 60°.

Reductions with $LiAlH_4$ (and $LiAl^2H_4$) were performed on 100–500-μg samples of material in ether for 10 min.

Oxidations with CrO_3/H_2SO_4 were carried out in acetone at 5–10° for 15 min.

Carboxylic acids were converted to their R-(+)-α-phenylethylamides by initial treatment (in toluene solution) with redistilled $SOCl_2$, and reaction of the acid chloride (without purification) with the chiral amine in toluene for 10 min.

Partial resolution of ibuprofen (cf. ref. 3)

(±)-2-(4-Isobutylphenyl)propionic acid was treated with (+)-α-phenylethylamine in ether. The precipitated salt was collected and recrystallised from acetone four times (m.p. 127–132°). The free acid was liberated by addition of HCl, and extracted into ether. The liberated acid had $[\alpha]_D$—47.4° (*c*, 1 g/100 ml in chloroform); GLC analysis (Table I) of the derived (+)-α-phenylethylamide indicated an optical purity of approximately 90%. For the parent compound, α-phenylpropionic acid, the elution order of α-phenylethylamides is RR before RS[5]. This would indicate that the resolved acid had the R-(−)-configuration.

Isolation of acidic metabolites

A 20-h urine sample was collected from a male volunteer who had ingested three 200-mg tablets of "Brufen". The urine was diluted with saturated brine and the

pH adjusted to 12 with 6 *M* aqueous NaOH. Non-acidic material was extracted with ether. The aqueous phase was acidified with 12 *M* HCl to pH 1, and extracted with ether–ethyl acetate; the extract was dried over Na_2SO_4 and evaporated at reduced pressure to yield a crude acidic fraction (900 mg). Initial purification was effected on a column (10 g) of silica gel; the solvent system used for elution was chloroform–methanol–acetic acid (80:20:5), and eluates were collected in 5-ml fractions. Portions of fractions 1–3 were further purified by preparative thin-layer chromatography (TLC) (mobile phase: chloroform–methanol–acetic acid, 20:1:0.5), methylated and examined by GLC and GLC–MS. We defer our report on later fractions.

The major component of fractions 1 and 2 was identified as 2-(4-isobutylphenyl)propionic acid, while fraction 3 contained four metabolites. Isolation of these metabolites was attempted using the lipophilic gel, Sephadex LH-20 (mobile phase: 1,2-dichloroethane–methanol, 7:3). Although partial separation of all four metabolites was achieved, only metabolites 2 and 4 were obtained in pure form. Further samples of metabolites were isolated as their methyl esters by preparative TLC (mobile phase: chloroform–ethyl acetate, 3:1); typical R_F values are given in Table II.

RESULTS

Enantiomeric composition of urinary 2-(4-isobutylphenyl)propionic acid

The amides of RS-2-(4-isobutylphenyl)propionic acid with R-(+)-α-phenylethylamine were completely resolved ($\Delta I = 35$) on a 5-m 1% OV-17 column (Table I). This allowed us to demonstrate that the enantiomers of 2-(4-isobutylphenyl)propionic acid were excreted in different proportions. "Unmetabolised" acid was isolated mainly in fraction 2 from the dry column and, after formation of amides with R-(+)-α-phenylethylamine, GLC showed that this consisted of 80% (+)-2-(4-isobutylphenyl)-propionic acid (Fig. 1). The principal MS characteristics of the amides are also contained in Table I. Identical mass spectra were obtained for the amides of the authentic and isolated acid. The fragmentations were similar to those observed for corresponding amides of α-phenylbutyric and α-phenylpropionic acids[5], C-acyl cleavage yielding

TABLE I

GAS–LIQUID CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR THE AMIDES OF 2-(4-ISOBUTYLPHENYL)PROPIONIC ACID WITH R-(+)-α-PHENYLETHYLAMINE

	$I^{1\%\ SE-30}_{230°}$	$I^{1\%\ OV-17}_{220°}$	*Mass spectral characteristics*	
			$M^{+\cdot}$	*Other ions (% abundance)*
Amide of (+)-2-(4-isobutylphenyl)propionic acid	2340	2660	309 (12)	161 (100); 105 (72); 162 (36); 119 (27); 117 (14)
Amide of (−)-2-(4-isobutylphenyl)propionic acid	2310	2625	309 (12)	161 (100); 105 (80); 162 (35); 119 (28); 117 (14);

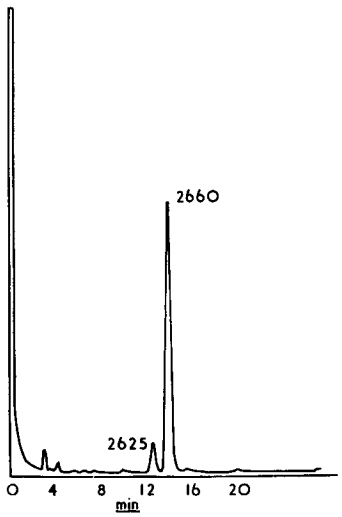


Fig. 1. GLC resolution of 2-(4-isobutylphenyl)propionic acid, isolated from urine, as amides with R-(+)-α-phenylethylamine. Column: 5 m, 1% OV-17, 245°.

m/e 161 characteristic of the acid and N-alkyl cleavage *m/e* 105 characteristic of the amine moiety.

The mass spectrum of ibuprofen methyl ester is shown in Fig. 2. Table III contains details of the mass spectra of the trimethylsilyl ethers of the products obtained by reduction of ibuprofen methyl ester with $LiAlH_4$ and $LiAl^2H_4$, respectively. These data were useful for comparison with spectra obtained from suspected metabolites.

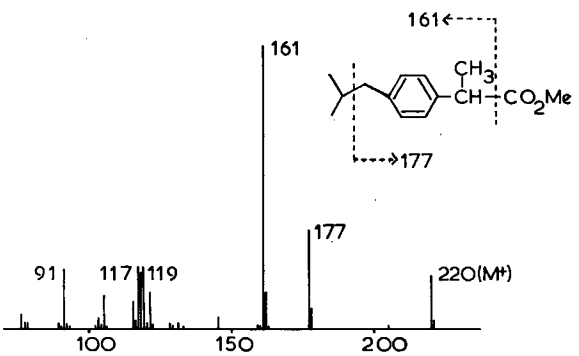


Fig. 2. Mass spectrum of 2-(4-isobutylphenyl)propionic acid methyl ester.

Characterisation of metabolites 1–4

Three hydroxylated metabolites and one dicarboxylic acid were characterised by GLC and GLC–MS as their methyl esters or methyl ester trimethylsilyl ethers (Table II). Two of the proposed structures (Fig. 3, Nos. 2 and 4) corresponded to known metabolites[3,4]. The structures were confirmed by microchemical transformations, monitored by GLC and GLC–MS (Table III). In the case of metabolites 2 and 4, isolation of 4–5 mg of purified material allowed examination by ^1H NMR spectrometry.

TABLE II

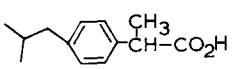
CHROMATOGRAPHIC DATA FOR DERIVATIVES OF 2-(4-ISOBUTYLPHENYL)PROPIONIC ACID AND ITS METABOLITES

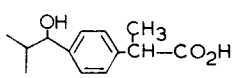
Solvent system for TLC: chloroform–ethyl acetate (3:1).

	R_F value	$I^{1\%\ OV\text{-}1}_{150°}$	$I^{1\%\ OV\text{-}17}_{170°}$	$I^{1\%\ QF\text{-}1}_{150°}$
2-(4-Isobutylphenyl)-propionic acid methyl ester	0.70	1515	1725	1800
Metabolite 1 methyl ester	0.50	1705	1950	2225
Metabolite 1 methyl ester trimethylsilyl ether	—	1700	1860	2135
Metabolite 2 methyl ester	0.56	1775	2080	2260
Metabolite 3 methyl ester	—	1780	—	—
Metabolite 3 methyl ester trimethylsilyl ether	—	1840	2025	—
Metabolite 4 methyl ester	0.34	1660	1935	2090
Metabolite 4 methyl ester trimethylsilyl ether	—	1780	1950	2080

Metabolite 1: 2,4'-(1-hydroxy-2-methylpropyl)phenylpropionic acid. The principal diagnostic ion (*m/e* 265) in the spectrum of the methyl ester trimethylsilyl ether of metabolite 1 (Fig. 4) was produced by cleavage adjacent to the secondary trimethylsilyloxy function. This was confirmed by a shift of 9 m.u. to *m/e* 274 on formation of the perdeuterated[6] trimethylsilyl ether. No molecular ion was observed. Loss of the carbomethoxy group produced the fragment at *m/e* 249, and, together with the loss of trimethylsilanol, that at *m/e* 159. Elimination of trimethylsilanol from the molecular ion itself was not observed, but an ion of low abundance at *m/e* 219 indicated the loss of a trimethylsilyloxy radical as expected from a benzylic trimethylsilyl ether. The abundant ion at *m/e* 133 was at first ascribed to an impurity retaining the unsubstituted isobutylphenyl moiety, but was still formed from a purified sample and remains unassigned pending further examination.

After reduction and trimethylsilylation, cleavage α to the secondary trimethylsilyloxy group was still a major mode of fragmentation.





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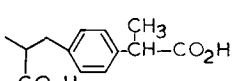

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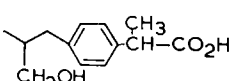

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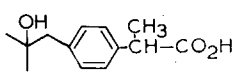

(4)

Fig. 3. Structures of ibuprofen and four of its urinary metabolites.

TABLE III

GAS–LIQUID CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR DERIVATIVES OF 2-(4-ISOBUTYLPHENYL)PROPIONIC ACID, AND OF SOME OF ITS METABOLITES

	Derivative	$I^{1\%\ OV\text{-}1}_{150°}$	*Mass spectral characteristics*	
			$M^{+\cdot}$	*Other ions (% abundance)*
2-(4-Isobutylphenyl)-propionic acid	Methyl ester	1515	220 (19)	161 (100); 177 (35); 117 (22); 119 (22); 91 (21)
	$LiAlH_4$ reduction product as TMS ether	1575	264 (7)	73 (100); 161 (98); 103 (95); 75 (38); 117 (18); 249 (18)
	$LiAl^2H_4$ reduction product as TMS ether	1565	266 (6)	161 (100); 73 (100); 105 (95); 75 (31); 251 (18); 119 (17)
Metabolite 1	Methyl ester	1705	236 (1)	193 (100); 105 (39); 133 (21); 194 (13); 134 (11)
	Methyl ester TMS ether	1700	308 (0)	265 (100); 73 (99); 133 (35); 266 (25); 75 (16); 159 (11); 74 (10); 117 (7); 206 (6)
	Methyl ester d_9-TMS ether	1700	317 (0)	82 (100); 274 (95); 133 (32); 275 (23); 83 (9); 159 (7); 215 (6)
	Methyl ester CMDMS ether	1930	342 (0.1)	299 (100); 79 (42); 301 (40); 107 (24); 300 (23); 133 (22)
	$LiAlH_4$ reduction product as TMS ether	1760	352 (0.1)	73 (100); 309 (67); 75 (31); 310 (19); 74 (6); 103 (6); 311 (6)
	$LiAl^2H_4$ reduction product as TMS ether	1755	354 (0.1)	73 (100); 311 (68); 75 (25); 312 (21); 74 (7); 105 (7); 313 (7)
	Ketone formed on oxidation of methyl ester	1705	234 (1)	191 (100); 103 (20); 104 (13); 192 (11); 77 (8); 78 (6); 132 (5); 175 (4)
Metabolite 2	Methyl ester	1775	264 (23)	205 (100); 145 (74); 177 (66); 117 (43); 91 (23); 118 (22); 121 (22); 204 (22)
	$LiAlH_4$ reduction product as TMS ether	1890	352 (0.1)	159 (100); 73 (78); 103 (53); 75 (28); 117 (15); 118 (15); 262 (15)
	$LiAlH_4$ reduction product as d_9-TMS ether	1890	370 (0.1)	159 (100); 82 (80); 112 (44); 81 (23); 271 (18); 118 (14); 117 (13)
	$LiAl^2H_4$ reduction product as TMS ether	1890	356 (0.1)	73 (100); 161 (95); 105 (70); 75 (35); 159 (32); 266 (16); 118 (16)
Metabolite 3	Methyl ester TMS ether	1840	308 (0.1)	159 (100); 218 (52); 73 (35); 117 (27); 131 (24)
	Methyl ester d_9-TMS ether	1840	317 (0.1)	159 (100); 218 (50); 82 (42); 131 (23); 117 (19)

TABLE III (*continued*)

	Derivative	$I_{150°}^{1\%\,OV\text{-}1}$	*Mass spectral characteristics*	
			$M^{+\cdot}$	*Other ions (% abundance)*
	Methyl ester CMDMS ether	2075	342 (0)	159 (100); 218 (54); 117 (35); 131 (20); 91 (17)
	$LiAlH_4$ reduction product as TMS ether	1890	352 (0.2)	73 (100); 159 (95); 103 (46); 75 (32); 117 (18); 262 (16); 118 (13)
	$LiAl^2H_4$ reduction product as TMS ether	1890	354 (0.2)	73 (100); 159 (89); 105 (62); 75 (36); 264 (18); 117 (17); 118 (15); 160 (15)
Metabolite 4	Methyl ester	1660	236 (0.1)	119 (100); 91 (95); 118 (86); 178 (55); 117 (20); 161 (15)
	Methyl ester TMS ether	1780	308 (0)	131 (100); 73 (70); 75 (17); 132 (11); 159 (5); 74 (4); 250 (4)
	Methyl ester d_9-TMS ether	1780	317 (0)	82 (100); 140 (63); 161 (27); 141 (20); 142 (10); 299 (7); 159 (6); 259 (4)
	Methyl ester CMDMS ether	2010	342 (0)	165 (100); 107 (46); 167 (36); 79 (30); 75 (25); 109 (17); 159 (16); 81 (13); 166 (13); 284 (10)
	$LiAlH_4$ reduction product as TMS ether	1835	352 (0.02)	131 (100); 73 (59); 75 (22); 72 (14); 132 (13); 247 (8); 103 (7)
	$LiAl^2H_4$ reduction product as TMS ether	1830	354 (0)	131 (100); 73 (58); 75 (20); 132 (14); 105 (6); 74 (5); 133 (5)

Metabolite 2: 2,4'-(2-carboxypropyl)phenylpropionic acid. The mass spectrum of metabolite 2 as the methyl ester gave a molecular ion of 264, corresponding to a dicarboxylic acid dimethyl ester (Fig. 5). The absence of hydroxyl groups was confirmed by the unchanged retention index and mass spectrum after treatment with trimethylsilylating reagents.

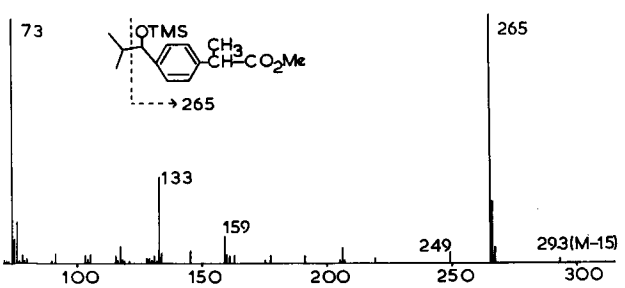

Fig. 4. Mass spectrum of metabolite 1 as its methyl ester trimethylsilyl ether.

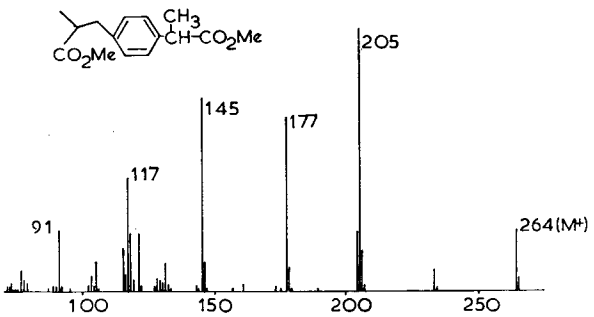

Fig. 5. Mass spectrum of metabolite 2 as its dimethyl ester.

The major fragments in the mass spectrum were due to the loss of a carbomethoxy group (*m/e* 205) and to the combined loss of carbomethoxy and $C_2H_4O_2$ groups (*m/e* 145). Reduction of metabolite 2 with $LiAlH_4$ (or $LiAl^2H_4$) and subsequent trimethylsilylation yielded a product of molecular weight 352 (or 356) thus confirming the presence of two acid moieties. The base peak (*m/e* 159) in the mass spectrum of this product was produced by a combination of two processes, *viz.* loss of trimethylsilanol (*m/e* 262) and elimination of the primary $[CH_2–OTMS]\cdot$ radical.

The structure of this metabolite was confirmed by ^1H NMR spectrometry of the methyl ester: δ, 7.15 (4H, q, $J = 8$ Hz) [aromatic –H]; 3.65 (3H, s) [original $-COOCH_3$]; 3.6 (3H, s) [new $-COOCH_3$]; 3.6 (1H, q, $J = 6$ Hz) [benzylic >CH–]; 2.8 (3H, m) [benzylic $-CH_2-$ and side-chain >CH–]; 1.5 (3H, d, $J = 6$ Hz) [$-CH_3$ coupled to benzylic >CH–]; 1.25 (3H, d, $J = 6$ Hz) [side-chain $-CH_3$].

Metabolite 3: 2,4'-(2-hydroxymethylpropyl)phenylpropionic acid. The mass spectrum of the methyl ester trimethylsilyl ether of metabolite 3 (Fig. 6) was characterised by the fact that none of the major ions contained the trimethylsilyloxy function, as shown by comparison with the spectrum of the perdeuterated trimethylsilyl ether or the chloromethyldimethylsilyl ether. The elimination of trimethylsilanol yielded an ion of 52% abundance, and together with loss of the carbomethoxy group gave the base peak at *m/e* 159. The ion at *m/e* 103, though of relatively low abundance (7%), was significant since it was shifted to *m/e* 112 in the spectrum of the perdeuterated trimethylsilyl ether; it thus corresponded to $CH_2{=}O^+–Si(CH_3)_3$, consistent with a primary hydroxyl function. The position of hydroxylation was confirmed by $LiAlH_4$

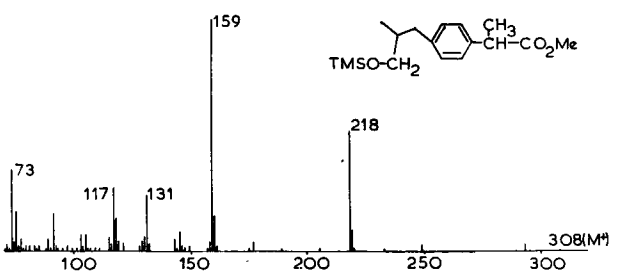

Fig. 6. Mass spectrum of metabolite 3 as its methyl ester trimethylsilyl ether.

reduction of the acid function; the trimethylsilyl ether of the resulting diol was identical to that obtained from metabolite 2 in both GLC and GLC–MS behaviour.

Metabolite 4: 2,4'-(2-hydroxy-2-methylpropyl)phenylpropionic acid. This was a hydroxylated metabolite, but the difficulty experienced in forming the trimethylsilyl ether suggested that the hydroxyl group was at the tertiary position in the isobutyl side-chain. In accordance with this, the mass spectrum of the methyl ester (Fig. 7) showed intense peaks at *m/e* 178 (cleavage α to the hydroxyl group with hydrogen transfer) and *m/e* 119 (additional loss of a carbomethoxy radical). A prominent ion at *m/e* 159 could be ascribed to elimination of water and a carbomethoxy radical. In the mass spectrum of the methyl ester trimethylsilyl ether, the base peak occurred at *m/e* 131, corresponding to $(CH_3)_2C{=}O^+{-}SiMe_3$. As expected, this changed to *m/e* 140 on formation of the perdeuterated trimethylsilyl ether, and to *m/e* 165 in the chloromethyldimethylsilyl ether. Even after reduction of metabolite 4 with $LiAlH_4$, and trimethylsilylation, this fragment remained the base peak.

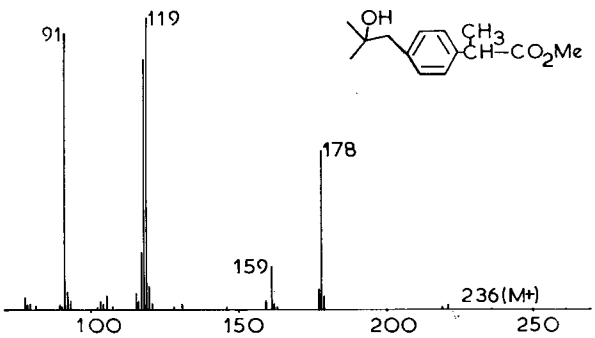

Fig. 7. Mass spectrum of metabolite 4 as its methyl ester.

The structure of the methyl ester of this metabolite was confirmed by ^1H NMR spectrometry: δ, 7.2 (4H, q, $J = 8$ Hz) [aromatic –H]; 3.7 (3H, s) [$-COOCH_3$]; 3.6 (1H, q, $J = 6$ Hz) [benzylic >CH–]; 2.75 (2H, s) [benzylic $-CH_2-$]; 1.5 (3H, d, $J = 6$ Hz) [$-CH_3$ coupled to benzylic >CH–]; 1.25 (6H, s) [side-chain $-(CH_3)_2C(OH)-$].

DISCUSSION

There are many instances in which enantiomeric forms of a drug differ markedly in biological activity: *d*- and *l*-"DOPA" [3-(3,4-dihydroxyphenyl)-α-alanine] are a classic example (*cf.* ref. 7 and references there cited). Where a drug is commonly administered in its racemic form, it is clearly important to establish the contributions of each enantiomeric form, and of their respective metabolites, to the pharmacological effects. Such knowledge is especially required for drugs that may be administered in relatively high dosage over a long term. Considerable attention has been given, for example, to the anticoagulant drug warfarin; this is normally administered in racemic form, but the S-isomer is the more potent enantiomer. Moreover, the drug undergoes stereoselective metabolic reduction, affording diastereomeric products that contribute to the total pharmacological response[8,9].

The anti-rheumatic drug ibuprofen is in widespread use for long-term maintenance therapy at a dosage of about 500 mg/day. The two human urinary metabolites hitherto identified[3,4] were both optically active, indicating stereoselectivity of metabolism. Two further metabolites of unspecified structure have been reported. The initial aims of our work have been to devise a rapid method, based on GC, for determining the enantiomeric composition of ibuprofen excreted in urine, and to establish the basic structures of additional metabolites. Attention will later be devoted to the stereochemical constitution of the metabolites, and finally it is hoped to devise a convenient analytical procedure for comparative clinical studies. With this last point in mind, all the results reported here were obtained from a single 20-h urine specimen.

The enantiomeric composition of ibuprofen samples was readily determined by means of the derived (+)-α-phenylethylamides, which were completely separated by GC. The significance of the increased proportion of (+)-enantiomer cannot be assessed until the configurational relationships between the drug and its other metabolites have been established.

The pattern of transformations of the isobutyl group in the oxidised metabolites was qualitatively parallel to that observed in the metabolism of the hypoglycaemic agent 2-*p*-methoxybenzenesulphonamido-5-isobutyl-1,3,4-thiadiazole[10], *viz.* hydroxylation at the three possible sites, and further oxidation of the primary alcohol to the carboxylic acid. The three isomeric hydroxy derivatives were readily distinguished by GC of their methyl esters, the order of elution being, as expected, tertiary OH < secondary OH < primary OH. Metabolic hydroxylation at the 2-position of an isobutyl group has also been observed for the psychotropic drug tetrabenazine[11].

The present results further illustrate the convenience of gas-phase methods for the micro-analytical characterisation of enantiomers[12]. The development and application of novel chiral reagents[13–16], particularly suited for GC and MS, is of great importance if the full potential of these techniques is to be realised.

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