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IN VIVO METABOLISM OF LIDOCAINE IN THE RAT

ISOLATION OF URINARY METABOLITES AS PENTAFLUOROBENZOYL DERIVATIVES AND THEIR IDENTIFICATION BY COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

The metabolism of a large dose (40 mg/kg intraperitoneally) of lidocaine (LIDO) in mature male Sprague–Dawley rats is described. Pentafluorobenzoyl chloride was used to derivatize the hydrolyzed urinary metabolites prior to extraction and analysis as pentafluorobenzoyl-derivatives by combined gas chromatography–mass spectrometry. Total ion and selected ion current (*m/z* 195; $C_6F_5CO^+$) traces were recorded and metabolites of LIDO were readily identified. Only one major metabolite, 3-hydroxy-N-(N-ethylglycyl)-2,6-xylidine, was excreted in urine. A new metabolite, 3-hydroxy-N-glycyl-2,6-xylidine was also present in significant amounts, as well as minor quantities of four oxygenated metabolites of N-(N-ethylglycyl)-2,6-xylidine. Other known metabolites of LIDO, including 3-hydroxylidocaine, were excreted in trace quantities. The results suggest that metabolism of LIDO in rats may be age- and/or dose-dependent.

INTRODUCTION

The metabolism of lidocaine (LIDO) has been studied extensively in vivo in various species [1–4]. The major metabolites in rats differ appreciably from those produced by man and other species. When LIDO (20 mg/kg per os) was administered to young (150 g) female Sprague–Dawley rats, the major urinary metabolites were found to be 3-hydroxylidocaine (3-OH-LIDO) (31.2% of administered dose), 3-hydroxy-N-(N-ethylglycyl)-2,6-xylidine (3-OH-MEGX) (36.9%), and 4-hydroxy-2,6-xylidine (4-OH-XYL) (12.4%). Minor urinary components were N-(N-ethylglycyl)-2,6-xylidine (MEGX) (0.7%), N-glycyl-2,6-xylidine (GX) (2.1%) and 2,6-xylidine (XYL) (1.5%), and traces (0.2%) of LIDO itself [1].

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The in vitro metabolism of LIDO in Wistar rats has also been investigated. Liver perfusion studies [5, 6] have again shown that the major metabolites in this system are 3-OH-LIDO and 3-OH-MEGX. A new in vitro metabolite, N-(N,N-diethylglycyl)-2-hydroxymethyl-6-methylaniline (LIDO-CH_2OH), the result of hydroxylation of a ring methyl group, has recently been identified in perfusion studies in liver from young Fischer 344 and Wistar rats [7, 8].

The reported method [1] for the analysis of in vivo metabolites of LIDO in rat urine utilized three separate analytical procedures and three different reagents for derivatization prior to gas chromatographic (GC) analysis on packed columns. The resulting GC peaks were broad and not completely resolved. Our current interest in the metabolism and pharmacokinetics of LIDO in different species encouraged us [9] to develop an extractive acylation procedure for the derivatization of LIDO metabolites in an aqueous medium followed by GC analysis and confirmation of structures by combined gas chromatography–mass spectrometry (GC–MS). Extractability of metabolites was improved by their derivatization in the aqueous medium with pentafluorobenzoyl chloride (PFBCl), after hydrolysis of conjugates. We now report our use of this procedure in a study on the metabolism of LIDO in Sprague–Dawley rats, and provide evidence that the rat produces more metabolites from LIDO than previously believed. The reported in vivo yield of 3-OH-LIDO in rats is questioned as a result of our studies.

The structures of all compounds are given in Fig. 1.

EXPERIMENTAL

Chemicals and reagents

Samples of LIDO, MEGX, GX, 3-OH-LIDO, 3-OH-MEGX and 4-OH-XYL were gifts from Astra Pharmaceuticals Canada (Mississauga, Canada). The syntheses of other metabolites will be reported elsewhere. PFBCl was purchased from Aldrich (Milwaukee, WI, U.S.A.). All solvents and reagents were obtained from reliable commercial sources. Where appropriate, solvents were distilled before use. Water was distilled and deionized (Milli-Q reagent water system, Millipore, Bedford, MA, U.S.A.).

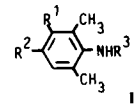
Animals

Male Sprague–Dawley rats (400–500 g) were administered lidocaine hydrochloride (40 mg/kg) intraperitoneally in sterile isotonic saline solution. The animals were housed singly in clear plastic metabolism cages, and urine was collected for 24 h, then frozen at −24°C until analyzed.

Apparatus

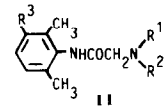
The gas chromatograph was a Hewlett-Packard (Palo Alto, CA, U.S.A.) 5730A equipped with a 15-mCi ^{63}Ni linear source for electron-capture detection (ECD), a 18740B capillary column controller and a 3390A integrator–recorder. The Reacti-Therm system was purchased from Pierce (Rockford, IL, U.S.A.) and the Savant Speed Vac concentrator–evaporator 100H from Emerston Instruments (Scarborough, Canada). All solvent evaporation procedures utilized this apparatus.

	R^1	R^2	R^3
a)	H	H	H
b)	H	OH	H
c)	H	H	COC_6F_5
d)	H	$OCOC_6F_5$	COC_6F_5
e)	OH	H	H
f)	$OCOC_6F_5$	H	COC_6F_5



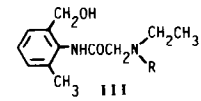
I

	R^1	R^2	R^3
a)	CH_2CH_3	CH_2CH_3	H
b)	CH_2CH_3	H	H
c)	H	H	H
d)	CH_2CH_3	COC_6F_5	H
e)	CH_2CH_3	H	OH
f)	CH_2CH_3	COC_6F_5	$OCOC_6F_5$
g)	CH_2CH_3	CH_2CH_3	OH
h)	CH_2CH_3	CH_2CH_3	$OCOC_6F_5$
i)	H	COC_6F_5	$OCOC_6F_5$
j)	CH_2CH_3	COC_6F_5	OH
k)	CH_2CH_3	COC_6F_5	$OCOCH_3$



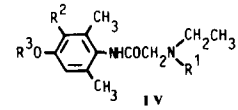
II

	R
a)	CH_2CH_3
b)	H
c)	COC_6F_5



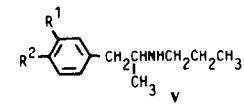
III

	R^1	R^2	R^3
a)	COC_6F_5	OCH_3	COC_6F_5
b)	H	OCH_3	H
c)	H	OH	H



IV

	R^1	R^2
a)	H	H
b)	H	OH
c)	OCH_3	OH
d)	OH	OH



V

Fig. 1. Structures of lidocaine, its metabolites and reference compounds.

Samples were mixed with an IKA-Vibrax-VXR multitube vortex mixer (Terochem, Edmonton, Canada) and centrifuged with a Microfuge B (Beckman, Palo Alto, CA, U.S.A.) or Dynac (Becton Dickinson, Parsippany, NJ, U.S.A.) bench top centrifuge. Electron-impact (EI) and chemical ionization (CI) (reactant gas isobutane) mass spectra were recorded by Dr. A.M. Hogg and his staff (Chemistry Department, University of Alberta), on a VG Micromass 7070E mass spectrometer linked to a Varian Vista 6000 GC and a PDP 11 data system (Analytech Instrumentation and Service, St. Laurent, Canada).

Chromatographic conditions

Chromatographic separation of LIDO metabolites was achieved on (a) a 30-m DB-1 capillary column (J & W Scientific, Palo Alto, CA, U.S.A.) with a column temperature of 180°C for 2 min, then to 280°C at 8°C/min, followed by 20 min at 280°C; or on (b) a 30-m DB-1701 capillary column (J&W Scientific) at 70°C for 2 min, then to 300°C at 6°C/min, followed by 8 min at 300°C. The injection

port and detector temperatures were set at 250°C and the column head pressure was maintained at 0.96 bar.

Pentafluorobenzoylation procedure

A portion (0.2 ml) of the 24-h rat urine sample was diluted with deionized, distilled water (0.3 ml) and 6 *M* hydrochloric acid (0.5 ml), and the flask containing the mixture was heated at 100°C for 15 min in the Reacti-Therm system to hydrolyze conjugated metabolites. The cooled solution was neutralized with 3 *M* sodium hydroxide then basified with solid potassium hydrogen carbonate (200 mg). To this solution were added 5 μl of PFBCl and 3 ml of toluene. The immiscible phases were vortexed for 30 min and centrifuged (Dynac) for 5 min at 3000 *g*. The organic layer was transferred to a small culture tube (100×13 mm) and evaporated to dryness. To the residue was added 300 μl of toluene and 600 μl of 1% ammonium hydroxide (to remove excess PFBCl). The phases were mixed for 30 s, then transferred to a 1.5-ml polyethylene microfuge tube and centrifuged briefly in the Microfuge B centrifuge. The organic layer was retained, and 1-μl samples were used for GC and GC–MS analyses.

RESULTS AND DISCUSSION

The identities of the metabolites of LIDO which gave rise to the GC peaks depicted in Fig. 2 were tentatively deduced by interpretation of the mass spectra obtained by scanning each peak. In those instances where authentic reference compounds were available, tentative identifications were confirmed by comparisons of retention times on the DB-1 GC column and mass spectra of the derivatized metabolites with those of similarly derivatized reference compounds which were analyzed by GC–MS under identical experimental conditions. Some diagnostic fragment ions were common to various spectra; their structures are given in Fig. 3. Mass spectral data are given in Table I and Fig. 4.

The total ion and selected ion (*m/z* 195) traces of the extract of rat urine after hydrolysis and treatment with PFBCl are shown in Fig.2, top and bottom traces, respectively. The time scan of these traces is 10.23–21.36 min after injection of a portion of the extract. The scans contain pentafluorobenzoylated (PFB) derivatives of amine and phenolic metabolites but exclude PFB derivatives of the minor metabolites XYL and MEGX which had retention times (t_R) of 5.78 and 9.49 min, respectively, in this GC system. The selected ion GC trace is of particular importance because all PFB-derivatized products are readily apparent in such a trace. The base peak in the spectra of the PFB metabolites, with two exceptions (PFB-XYL and PFB-GX), is the $C_6F_5CO^+$ (*m/z* 195) fragment ion. LIDO and LIDO-CH_2OH could not be detected by this procedure because neither compound undergoes derivatization with PFBCl in aqueous solution. The mass spectrum of each peak was recorded; repetitive scans of the broad peaks (Fig. 2, peaks 133–145 and 403) were made. Those GC peaks which gave spectra containing ions of *m/z* 195 and 167 ($C_6F_5CO^+$ and $C_6F_5^+$, respectively) and were metabolites of LIDO, are identified by asterisks in Fig. 2.

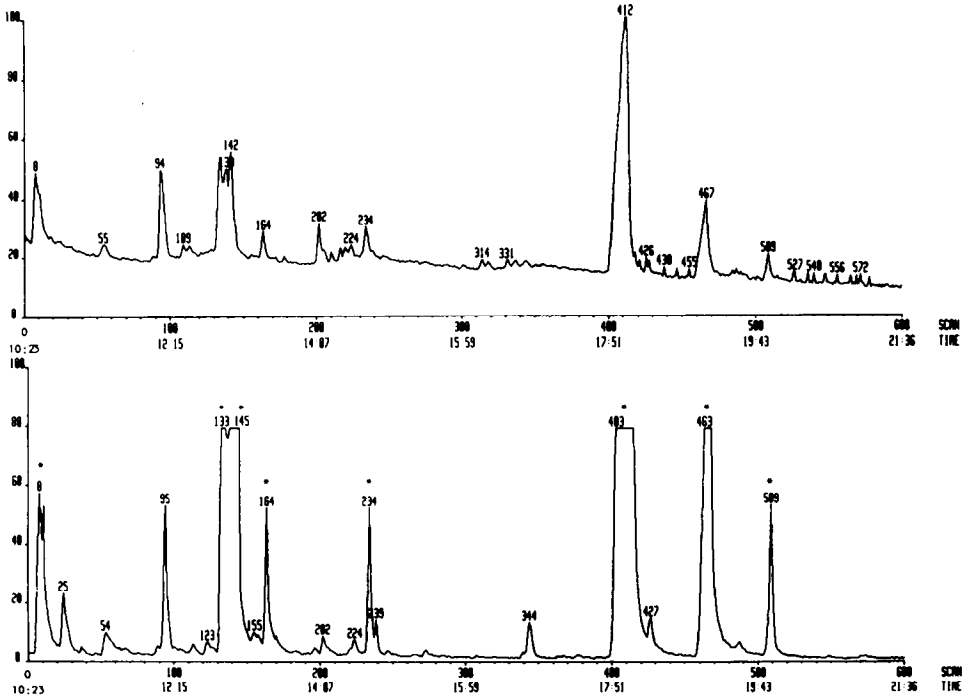


Fig. 2. Total ion (top) and selected ion *m/z* 195 (bottom) traces on a DB-1 capillary column of a PFB urine extract from rats that had received lidocaine (* = metabolite of lidocaine).

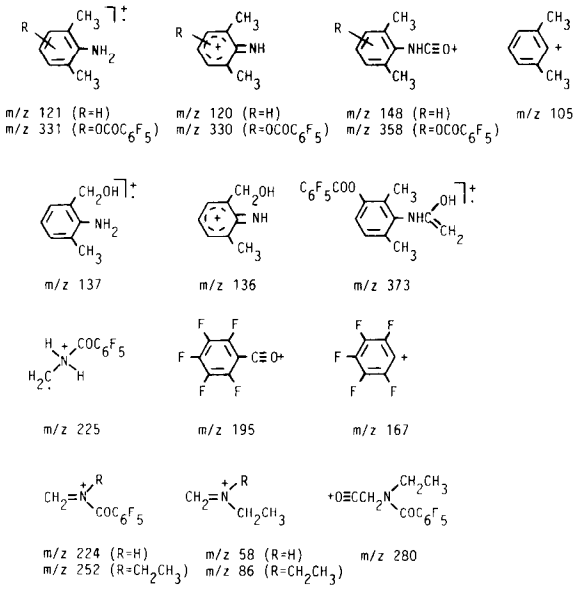


Fig. 3. Diagnostic fragment ions in the EI mass spectra of PFB metabolites of lidocaine.

TABLE I

MASS SPECTRAL DATA

Compound	*m/z*	Relative abundance (%)	Identity
Ic	315	46.9	M^+
	300	7.6	$[M-CH_3]^+$
	298	9.3	$[M-OH]^+$
	195	47.4	–★
	167	9.2	–
	120	100	–
IId	400	8.8	M^+
	280	23.8	–
	252	36.9	–
	195	100	–
	167	11.9	–
	121	28.9	–
	120	17.1	
IIh★★	444	2.2	M^+
	443	2.4	–
	429	4.7	$[M-CH_3]^+$
	427	3.3	$[M-OH]^+$
	357	2.4	$[ArN{=}C{=}O]^+$
	331	2.9	$[ArNH_2]^+$
	330	3.5	$[Ar{=}NH]^+$
	195	90.5	–
	167	18.7	–
	87	29.0	–
	86	100	–
	72	27.1	$[m/z\ 87-CH_3]^+$
	58	82.9	$[m/z\ 86-CH_2{=}CH_2]^+$

★For identities of these ions, see Fig. 3.

★★Ar = 3-C_6F_5COO-2,6-$(CH_3)_2$-C_6H_2–.

The presence of four known urinary metabolites of LIDO in the PFB-derivatized extract was readily confirmed.

XYL (Ia). The GC peak corresponding to the PFB derivative (Ic) of this minor metabolite (t_R 5.78 min) is not included in Fig. 2. Its relatively simple EI spectrum contained diagnostic ions of *m/z* 315 (M^+), 300 $(M-15)^+$, 298 $(M-OH)^+$, 195, 167 and 120. The structures of the last three ions are given in Fig. 3. The ion of *m/z* 120 is the base peak and is the result of the expulsion of a C_6F_5CO radical from the molecular ion. The GC and MS properties of a PFB-derivatized authentic sample of XYL were identical to those of the derivatized metabolite.

MEGX (IIb). This was also a minor metabolite. The GC peak of its PFB derivative (IId, t_R 9.49 min) is not included in Fig. 2. The EI mass spectrum of this peak had diagnostic ions of *m/z* 400 (M^+), 280, 252, 195, 167, 121 and 120. Its CI mass spectrum had an MH^+ peak at *m/z* 401 (30% relative abundance) and an $(MH-HF)^+$ fragment of *m/z* 381 (100%). Appropriate structures for EI fragment ions of *m/z* 280, 252, 195, 167, 121 and 120 are given in Fig. 3. The ions of

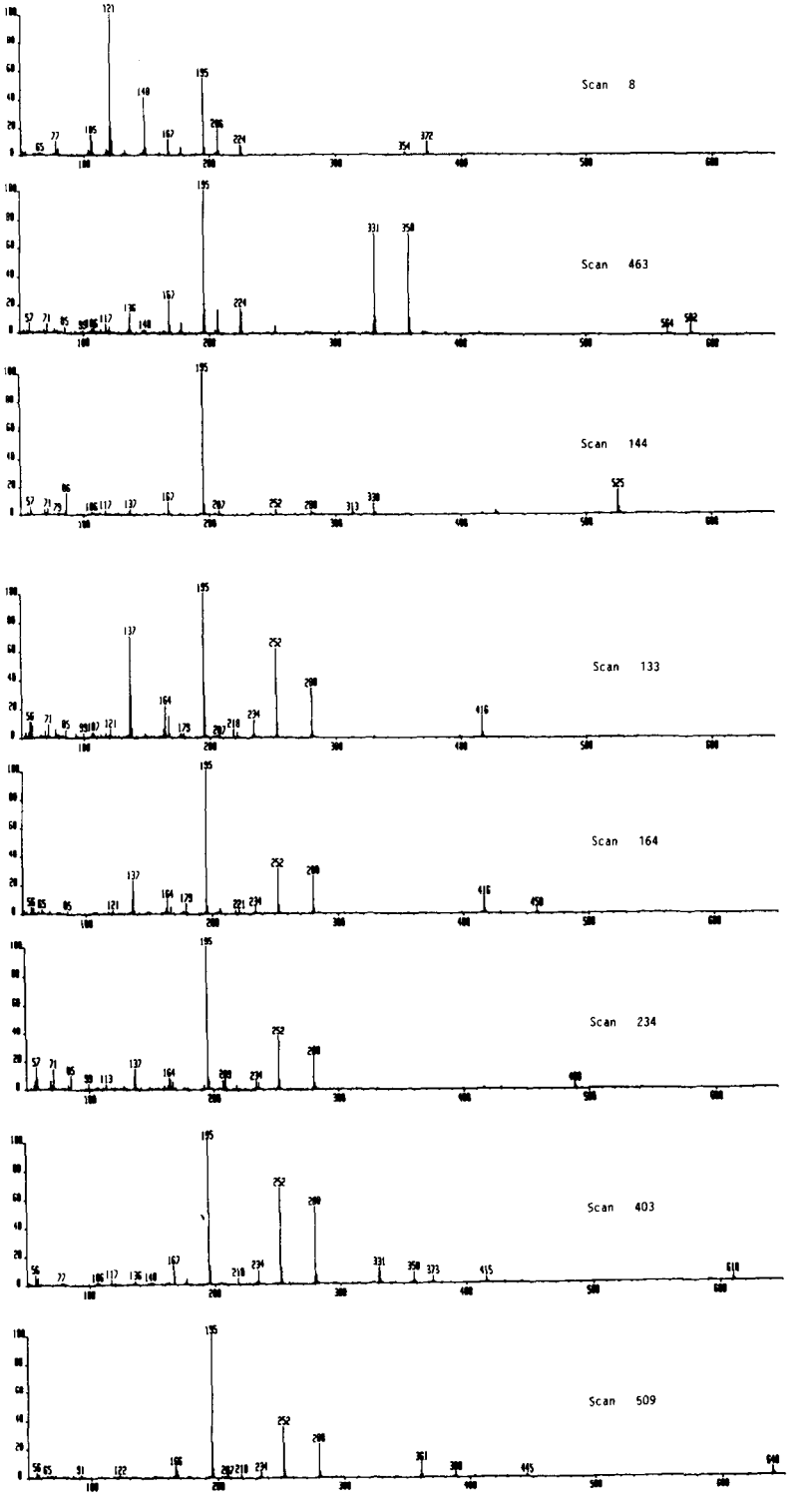


Fig. 4. EI mass spectra of lidocaine metabolites as their PFB derivatives.

m/z 121 and 120 are particularly diagnostic; their presence in the EI spectrum of this derivatized metabolite confirms that the compound contains an intact 2,6-dimethylanilino moiety. These data suggested that this compound was the N-PFB derivative of MEGX (IId). An authentic sample of MEGX was also pentafluorobenzoylated. The product had GC and MS properties identical to those of the derivatized metabolite [9].

GX (IIc). The peak at 10.32 min (scan 8, Fig. 2, bottom trace) was deduced to be that of the N-PFB derivative of GX. Its EI mass spectrum (Fig. 4) contained diagnostic ions of *m/z* 372 (M^+), 354, 225, 224, 195, 167, 148, 121, 120 and 105. With the exception of the ion of *m/z* 354, appropriate structures for all of these ions are given in Fig. 3. The ion of *m/z* 354 is the result of the expulsion of a water molecule from the molecular ion [9]. When an authentic reference sample of GX was pentafluorobenzoylated, the product had MS and GC properties identical to those of peak 8. Both spectra contained an ion of *m/z* 206 which is believed to be derived from the *m/z* 354 fragment ion by the expulsion of a $C_6H_3(CH_3)_2NHCO$ radical, with rearrangement [9].

3-OH-MEGX (IIe). Repetitive scans of the most abundant GC peak, at 17.74 min (Fig. 2, bottom trace), showed that it contained only one product. Its EI mass spectrum (Fig. 4; scan 403) is consistent with this compound being N,O-di-PFB-3-OH-MEGX (IIf). 3-OH-MEGX is the known major metabolite of LIDO in rats [1]. Diagnostic ions in the spectrum were of *m/z* 610 (M^+), 415, 373, 358, 331, 330, 280, 252, 195 and 167. Structures for most of these ions can be readily deduced by comparisons with the spectrum of derivatized MEGX (IId); they are given in Fig. 3. The ion of *m/z* 415 is clearly the result of the expulsion of a C_6F_5CO radical from the molecular ion of IIf, while the ion of *m/z* 373 (Fig. 3) could be the result of a McLafferty rearrangement. The GC properties and mass spectrum of the PFB derivative of an authentic synthetic sample of 3-OH-MEGX were identical to those of the derivatized metabolite. The isomeric di-PFB derivative of 4-OH-MEGX, described in our previous study [9], had an EI mass spectrum virtually identical to that of IIf but had a different retention time (18.20 min). The identity of this metabolite of LIDO as 3-OH-MEGX is therefore unequivocal.

Other metabolites of lidocaine were also identified. Peak 133–145 (t_R 12.53–13.10 min) is a strong peak in the GC trace (Fig. 2, bottom trace). As 3-OH-LIDO (IIg) and 4-OH-XYL (Ib) are claimed to be major metabolites of LIDO in the rat [1, 7] information on the GC behavior of the PFB derivatives of both metabolites was required. Authentic samples of Ib and IIg were synthesized and derivatized with PFBCl. When chromatographed under the same conditions as those used to produce Fig. 2, the synthetic PFB derivatives of IIg and Ib (IIh and Id, respectively) had virtually identical retention times (13.00–13.10 min). This observation suggested that the broad peak at 12.53–13.10 min (scans 133–145) was a mixture of IIh and Id. When this peak was repeatedly scanned, however, it was apparent that it contained very little of compound IIh. The EI mass spectrum of the synthetic sample of IIh (see Experimental) was consistent with its structure and contained diagnostic ions of *m/z* 86 (base peak) and 58, (structures given in Fig. 3). None of the scans 133–145 was that of compound

IIh. One scan (144) did contain an ion of *m/z* 86 but it was of low abundance, and its presence indicated only that a metabolite of LIDO which retained the diethylamino group was present in low concentration in this GC peak. It is clear that 3-OH-LIDO is not a significant in vivo metabolite when LIDO is administered at high doses to male Sprague–Dawley rats.

Repetitive scanning of peak 133–145 revealed that it contained at least two components, one of which was a new metabolite. The scan of the latter portion of this broad peak produced a spectrum (Fig. 4; scan 144) which was virtually superimposable on that of an authentic sample of N,O-bis-pentafluorobenzoyl-4-hydroxy-2,6-xylidine (di-PFB-4-OH-XYL; Id), except for the presence of the ion of low abundance at *m/z* 86. Diagnostic ions in this spectrum were of *m/z* 525 (M^+), 330 $(M-COC_6F_5)^+$, 195 and 167 (Fig. 3). The authentic sample of di-PFB-4-OH-XYL also had GC properties identical to those of the derivatized metabolite. Identification of this metabolite of LIDO as 4-OH-2,6-XYL (Ib) was indicated. However, when a synthetic sample of the isomer, 3-OH-XYL (Ie) was obtained and converted to the di-PFB derivative (If) with PFBCl, this derivative (Ie) had a GC retention time on the DB-1 column and a mass spectrum virtually identical to those of Id. Clearly, peak 133–145 contained 4-OH-XYL and/or 3-OH-XYL. Studies to resolve this equivocal conclusion were required.

PFB derivatives of N-monodeethylated metabolites of LIDO are readily identified by MS. MEGX is the prototype molecule. Its EI mass spectrum has three particularly diagnostic ions of *m/z* 280, 252 and 234; the structures of two of these ions are provided in Fig. 3; the other ion (*m/z* 234) is the result of the expulsion of a water molecule from the ion of *m/z* 252 [9]. Any PFB-derivatized metabolite of LIDO which gives a mass spectrum that contains these three ions can be identified as one of general structure $ArNHCOCH_2N(CH_2CH_3)COC_6F_5$, i.e. an aromatic ring metabolically modified MEGX molecule which retained an intact N-ethyl-N-PFB-glycyl side-chain. Four metabolites of LIDO [peaks 133, 164, 234 and 509 (Fig. 2, bottom trace)] clearly possess this general structure.

Peak 133 (t_R 12.53 min). Scanning of the early part of the broad GC peak (133–145), gave an EI mass spectrum which contained diagnostic ions of *m/z* 416, 280, 252, 234, 195, 167, 164, 137, 136, 121 and 105 (Fig. 4). The presence of the ions of *m/z* 280, 252 and 234, and a direct comparison of this spectrum with that of PFB-MEGX, indicated that peak 133 contained a monooxygenated derivative of MEGX and that the oxygen atom was located somewhere in the 2,6-dimethylanilino portion of the molecule.

This metabolite was initially deduced to be the result of oxidation of a ring-methyl group of MEGX (i.e. structure IIIa) for a variety of reasons: (i) an alcohol hydroxy group would not be pentafluorobenzoylated during the derivatization procedure in the aqueous medium, whereas a phenolic group, if present, would undergo derivatization (cf. peak 403); (ii) ring-methyl hydroxylated LIDO (IIIb) has recently been isolated [7, 8] as a metabolite of LIDO in a rat liver perfusion model; (iii) the structurally similar antiarrhythmic drug, mexiletine, is metabolized by ring-methyl group oxidation [10]; (iv) the ions of *m/z* 120 and 121 in the mass spectrum of PFB-MEGX are replaced with ions of *m/z* 136 and 137 (Fig.

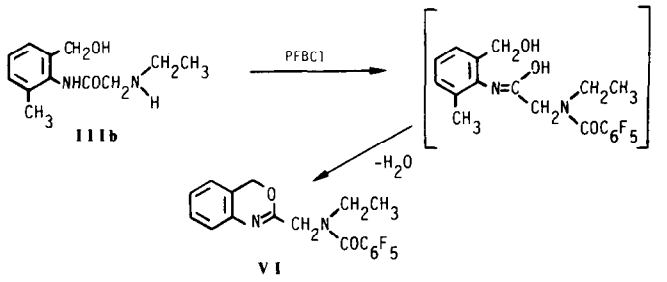


Fig. 5. Cyclization of the PFB derivative of a ring-methyl hydroxylated derivative of lidocaine.

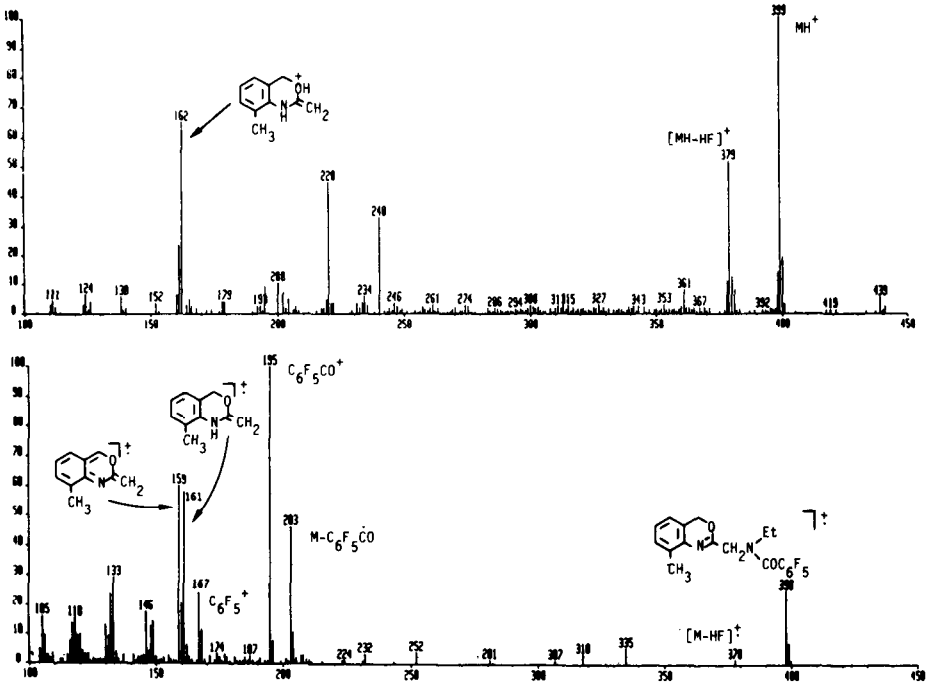


Fig. 6. The CI (top) and EI (bottom) mass spectra of a cyclized ring-methyl hydroxylated derivative of lidocaine. Identities of diagnostic ions are provided.

3) in the spectrum of this PFB-derivatized metabolite; (v) the presence of ions of *m/z* 164 and 121 in the spectrum is also compatible (Fig. 4) with the derivatized metabolite's structure being IIIc. This derivative, therefore, was tentatively identified as N-(N-ethyl-N-PFB)glycyl-2-hydroxymethyl-6-methylaniline (IIIc).

An authentic sample of IIIb was synthesized and then derivatized with PFBCl. The product was not identical to the derivatized metabolite. It had a different GC retention time (11.94 min) on the DB-1 column and a different mass spectrum. Synthetic IIIb underwent an interesting dehydration and cyclization reaction when reacted with PFBCl (Fig. 5). The product (VI) gave a mass spectrum which contained diagnostic ions of *m/z* 398 (M^+), 203 ($[M-COC_6F_5]^+$), 195 and 167 (Fig. 3), 161 and 159. The last two ions are presumably formed as shown

in Fig. 6. The CI mass spectrum had appropriate ions of *m/z* 399 (MH^+), 379 ($[MH-HF]^+$) and 162 (Fig. 6). This dehydration reaction during derivatization with PFBCl has potential importance. Reaction with PFBCl may represent a general method of identifying ring-methyl hydroxylated metabolites of LIDO and structurally related compounds.

Since the PFB-derivatized LIDO metabolite (t_R 12.53 min) and VI were not identical, the alternative possibility that the derivative was the mono-PFB derivative, 3-OH-N-PFB-MEGX (IIj), was considered. Such a product could be the result of (i) incomplete pentafluorobenzoylation of 3-OH-MEGX, (ii) partial hydrolysis of the di-PFB derivative (IIf) during the derivatization procedure, or (iii) hydrolysis of an O-acylated metabolite of 3-OH-MEGX during the ammonia treatment in the derivatization procedure. Attempts to partially pentafluorobenzoylate 3-OH-MEGX were unsuccessful; the di-PFB product (IIf) was always obtained, and the O-PFB group was stable to treatment with ammonia. It appears that the formation of this mono-PFB derivative of 3-OH-MEGX may be the result of hydrolysis of metabolically produced O-acyl derivatives of 3-OH-MEGX (see peaks 164 and 234, and Fig. 4). Further study on this topic is required.

Peak 164 (t_R 13.31 min). The EI mass spectrum of this peak (Fig. 4) was virtually identical to that of scan 133 except that it contained an ion of low abundance of *m/z* 458, in addition to all the diagnostic ions identified for peak 133. The compounds of scans 133 and 164 are clearly structurally related, and both are derivatives of MEGX. Although other interpretations are possible, a tentative explanation of the mass spectral data is that peak 164 contains the acetate derivative (IIk) of the compound in peak 133.

Peak 234 (t_R 14.48 min). This peak also contains a PFB-derivatized metabolite of LIDO, which may be related in structure to those in peaks 133 and 164. The presence of ions of *m/z* 280, 252 and 234 (Fig. 3) in its EI mass spectrum (Fig. 4) is consistent with a structure which contains an (N-ethyl-N-PFB)glycyl moiety. The formation of ions of *m/z* 164, 137 and 136 indicates that the compound in peak 234 possesses a hydroxylated 2,6-methylanilino group, or such a unit is produced during the mass fragmentation process. Peak 234, like 164, appears to contain an acyl derivative of 3-OH-MEGX. Further conjecture on the structure of this LIDO metabolite is not warranted.

Peak 509 (t_R 19.53 min). Interpretation of the EI mass spectrum (Fig. 4) of this GC peak indicates that it is the di-PFB derivative of a new, though minor metabolite of LIDO. A comparison of its mass spectrum with that of peak 403 (N,O-di-PFB-3-OH-MEGX) clearly illustrates that both compounds are structurally closely related. Ions common to both spectra are of *m/z* 280, 252, 234, 218, 195 and 167. A comparison of these spectra over the *m/z* 300–650 mass range reveals that they are again almost identical except that each fragment ion in the spectrum of peak 403 (*m/z* 610, 415, 373, 358 and 331) is displaced by 30 a.m.u. to *m/z* 640, 445, 403, 388 and 361, respectively, in the spectrum of 509. An appropriate interpretation of this observation is that peak 509 contains a ring-methoxylated analogue of 403.

While other isomers are possible, it is tempting to speculate that the compound in peak 509 possesses structure IVa, and that the minor metabolite of LIDO is 4-

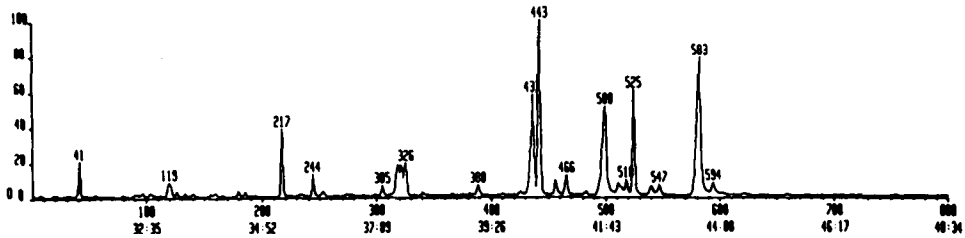


Fig. 7. Total ion trace on a DB-1701 capillary column of a PFB urine extract from rats that had received lidocaine.

hydroxy-3-methoxy-MEGX (IVb). Such a conclusion would be consistent with our previous observation [11] on the metabolism of N-*n*-propylamphetamine (Va) in the rat. Two phenolic metabolites obtained in that study were shown to possess structures Vb and Vc, and it was concluded that the intermediate metabolite in the formation of Vc was the catecholamine (Vd) whose conversion to Vc was catalyzed by catechol-O-methyltransferase (COMT). It is possible that LIDO is also metabolized to a small extent to 3,4-dihydroxy-MEGX (IVc) and this catechol is further metabolized by COMT to the 4-hydroxy-3-methoxy analogue (IVb). This possibility is being investigated further.

Peak 463 (t_R 19.05 min). EIMS scanning of this peak (Fig. 4) indicated that it contained a single compound and new metabolite of LIDO. Its mass spectrum contained diagnostic ions of *m/z* 582, 564, 358, 331, 225, 224, 195, 167 and 136. The ion of *m/z* 582 was the molecular ion; a CIMS scan of this peak contained ions of *m/z* 583 (MH^+; 88% relative abundance) and 565 ($[MH-H_2O]^+$) (8%). The fragment ions are readily identified (Fig. 3). Peak 463 obviously contains a di-PFB derivative of 3- or 4-OH-GX. Two fragment ions are of particular assistance in deducing that this metabolite is a di-deethylated derivative of LIDO. The ion of *m/z* 225 had already been identified (Fig. 3) and is also present in the spectrum of PFB-derivatized GX. The remaining diagnostic ion, *m/z* 564, results from the expulsion of a water molecule form the molecular ion. The same fragmentation pathway was observed in the spectrum of N-PFB-GX (Fig. 4) [9].

An authentic sample of 4-OH-GX was prepared, but various attempts to prepare 3-OH-GX have been unsuccessful. The di-PFB derivative of the former isomer was obtained. Its mass spectrum was virtually identical to that of the derivatized metabolite (peak 463), but its retention time on the DB-1 GC column (19.75 min) was significantly different. From this evidence, it is concluded that peak 463 contains the di-PFB derivative of 3-OH-GX, a new metabolite of LIDO in the rat. Such a metabolite is not unexpected in view of the fact that LIDO is known to be metabolized to 3-OH-LIDO and 3-OH-MEGX in that species.

The complexity of GC peak 133–145 suggested that a better resolution was required. Various GC conditions and columns were investigated and acceptable resolution was eventually obtained on a DB-1701 capillary column (Fig. 7). In this figure the peaks eluting between 39.26 and 44.00 min correspond to those in Fig. 2 (bottom trace) which elute between 12.30–13.45 min. The broad peak (133–145) was resolved into four peaks 437, 443, 525, and 583; Fig. 7); peak 500

in Fig. 7 corresponds to peak 164 in Fig. 2 (bottom trace). The mass spectra of all the four peaks in Fig. 7, referred to above, were interpreted and, where possible, compared to the spectra of PFB-derivatized synthetic reference samples. In this way, the four peaks were deduced to be the PFB derivative of 3-OH-LIDO (437; IIh), the di-PFB derivative of 3-OH-XYL (443; If), the di-PFB derivative of 4-OH-XYL (525; IId) and the N-mono-PFB derivative of MEGX (583; IIj).

CONCLUSION

PFBCl is a versatile and relatively new reagent which reacts in aqueous solutions with phenols to give PFB esters, and with primary and secondary amines to give PFB amides, but not with alcohols [12, 13]. The initial objective of the study now reported was to investigate the usefulness of pentafluorobenzoylation for the identification of phenolic and N-dealkylated metabolites of LIDO in the urine of rats. A major fragment ion in the EI mass spectra of PFB esters and amides is the ion of *m/z* 195 (Figs. 3 and 4). We chose to detect the GC peaks of hydrolyzed rat urinary metabolites of LIDO by MS operated in the selected ion mode, and we selected the *m/z* 195 ion for this purpose. In the resulting chromatogram (Fig. 2, bottom trace), only peaks which contained PFB-LIDO metabolites and PFB derivatives of endogenous compounds are displayed. The trace contains few interfering peaks; this is of value in the identification of metabolites. EI mass spectra of the eluting peaks were obtained by scanning over the *m/z* 50–650 mass range (Fig. 2). The results were surprising. In contrast to the results obtained by other investigators [1], only one major metabolite (3-OH-MEGX) was excreted in rat urine. Relatively minor quantities of the expected PFB derivatives of known phenolic and primary and secondary amine metabolites of LIDO (i.e. XYL, MEGX, GX, 4-OH-XYL and 3-OH-MEGX) were also found. In addition, at least seven new PFB-derivatized metabolites of LIDO were also detected, and one metabolite, 3-OH-LIDO, previously claimed [1] to be a major urinary metabolite of LIDO in rats, was found by this analytical procedure to be present in only trace amounts.

Appropriate structures for the seven new metabolites of LIDO were deduced by interpretation of their mass spectra. Three of them have been unequivocally identified: trace amounts of 3-OH-XYL and 4-OH-LIDO and appreciable quantities of 3-OH-GX. The deductions on the structures of the other four minor metabolites (GC peaks 133, 164, 234 and 509) are equivocal and will remain so until authentic samples of these proposed new metabolites are synthesized and their GC and MS properties are compared with those of the metabolites. What is clear, however, is that these four new metabolites are derivatives of MEGX. The EI mass spectra of all four metabolites contain fragment ions of *m/z* 280, 252 and 234 (Figs. 3 and 4) which are diagnostic of derivatized metabolites that contain a $-COCH_2N(CH_2CH_3)COC_6F_5$ moiety.

Repeat experiments in other mature Sprague–Dawley rats have confirmed these observations. In our experiments, the dose of LIDO administered to rats was higher than that previously given by other investigators [1], and older rats were employed in our study. It has been demonstrated by others [7, 8] that the in vitro metabolism of LIDO in two species of rats is influenced by the age of the rats. It

is conceivable that in vivo yields of 3-OH-LIDO and 4-OH-XYL may depend on the dose of LIDO administered, its route of administration and/or on the age of the rats used in the study. Further studies on this topic are in progress.

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