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DEGRADATION OF PERFLUOROACYL DERIVATIVES OF TOCAINIDE AND SOME OF ITS ANALOGUES IN THE PRESENCE OF AN EXCESS OF ANHYDRIDE REAGENT*

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SUMMARY

The perfluoroacylation of tocainide has been studied. The initially formed perfluoroacylamides were found to be degraded by an excess of perfluoroacyl anhydride. The reaction of tocainide with heptafluorobutyric anhydride gave similar results in six different solvents. Comparable amounts of decomposition occurred with trifluoroacetyl and pentafluoropropionyl anhydrides as reagents. Six new products were separated by gas chromatography and their structures tentatively assigned based on comparison with a deuterated analogue and mass spectral analysis including high resolution measurements: dehydrated and dehydrogenated heptafluorobutyryl (HFB)-tocainide, "apparent" pyruvic xylidide which still retains the perfluoro group, dehydrated HFB-tocainide and dehydrogenated HFB-tocainide. In the presence of water, these compounds could be quantitatively converted into HFB-tocainide with loss of tocainide's chirality. Studies with some tocainide analogues having a primary amino group and a secondary amide demonstrated that these functional groups are required to give labile HFB derivatives. On the contrary, stable derivatives of tocainide were formed with heptafluorobutyryl chloride and N-methyl-bis(heptafluorobutyramide).

INTRODUCTION

Gas chromatographic analysis of tocainide (Fig. 1A) is commonly performed

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A)

CH$_3$ CH$_3$
NHCOCHNH$_2$
CH$_3$

HFB | 0.01
anh | %

B)

CH$_3$ CH$_3$
NHCOCHNH-COC$_3$F$_7$
CH$_3$
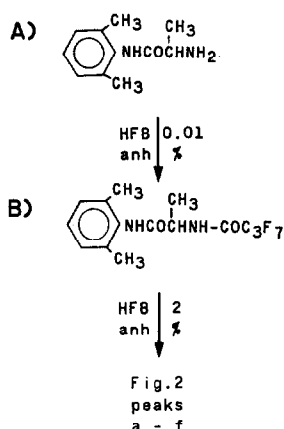
HFB | 2
anh | %

Fig.2
peaks
a - f

Fig. 1. Scheme for the reaction of tocainide (A) with HFBA leading to HFB-tocainide (B) and degradation of this derivative.

after its perfluoroacylation with heptafluorobutyric anhydride (HFBA) to form the heptafluorobutyryl (HFB) derivative (Fig. 1B)[1-3]. Perfluoroacylation of tocainide in toluene proceeds smoothly only with a reagent concentration of about 0.01% (v/v)[1]. The absolute yield in the derivatization step was 92% as determined with the synthetic HFB-tocainide derivative as a reference. Higher concentrations of anhydride, however, resulted in pronounced degradation of the originally formed derivative (Fig. 2)[1], *e.g.*, only 50% of HFB-tocainide remains after 30 min at 40°C and peaks with shorter gas chromatographic (GC) retention times are produced with 1% of anhydride.

The objectives of the present study were to determine the mechanisms involved in the degradation of the HFB-tocainide derivative and the structures of the products formed.
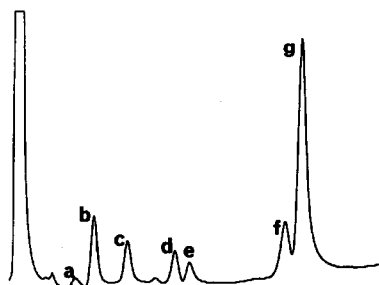
Fig. 2. Gas chromatogram of HFB-tocainide and its degradation products. Column: 3% OV-17, temperature raised from 100°C at 10°/min. Peaks: a = HFB-xylidine; b = dehydrated and dehydrogenated HFB-tocainide; c, d = dehydrated HFB-tocainide; e = pyruvic xylidide; f = dehydrogenated HFB-tocainide and g = HFB-tocainide (retention time 7.7 min).

EXPERIMENTAL

Apparatus
        Gas chromatography. A Varian 3700 gas chromatograph equipped with a therm-

ionic (nitrogen-selective) detector was used. The glass column (120 × 0.2 cm I.D.) was filled with 3% OV-17 on Gas Chrom Q (100–120 mesh). The flow-rate of nitrogen carrier gas was 30 ml/min. The temperatures of the injector and the detector were 250 and 300°C, respectively. The temperature of the column oven was programmed from 100 to 200°C at 10°C/min. A fused-silica capillary column (25 m × 0.32 mm) coated with 5% phenyl silicone was used. The inlet pressure of the carrier gas was 100 kPa, and the temperature of the column oven was programmed at a rate of 10°C/min from 70°C. Enantiomeric separation[1] was performed on a Chirasil-Val® column with a flame ionization detector. The peak areas were evaluated by a 3390A Hewlett-Packard integrator.

Mass spectrometry. Mass spectra were recorded in a Finnigan MAT 44 S instrument equipped with a Varian 3700 gas chromatograph. The fused-silica capillary column described above was connected to the mass spectrometer by an open split interface. Mass spectra were recorded under electron impact (70 eV) or chemical ionization (methane) conditions and were acquired by a Finnigan MAT SS 200 data system, followed by normalization and background subtraction. High-resolution (5000 by 10% valley definition) mass spectra of the derivatives were recorded in a VG 7070 instrument after separation on a DB-5 fused-silica capillary column (25 m × 0.3 mm I.D.). The elemental compositions of the ions were calculated by a VG Model 2035 data system.

Liquid chromatography. The liquid chromatographic system consisted of a Beckman 110A pump, a Rheodyne 7010 injection valve (loop volume 110 μl), a column (stainless steel, 150 × 4.5 mm) filled with 5-μm LiChrosorb Si 60 (Merck) and a Waters 440 detector. The flow-rate of the mobile phase was 1 ml/min and the absorbance of the eluent was monitored at 254 nm. The mobile phase was 5% 2-propanol, 0.1% water and 0.1% acetic acid in hexane (system A), or 10% tetrahydrofuran in hexane (system B).

Reagents and chemicals

The reagents were purchased from the following suppliers: trifluoroacetic (Fluka, Buchs, Switzerland), pentafluoropropionic (Reagenta, Uppsala, Sweden) and heptafluorobutyric anhydrides (Regis, Morton Grove, IL, U.S.A.); heptafluorobutyryl chloride (Fluorochem, Glossop, U.K.), N-methyl-bis(heptafluorobutyramide) (Regis) and heptafluorobutyric acid (Pierce, Rockford, IL, U.S.A.).

The following substances were synthesized by the Department of Organic Chemistry, AB Hässle: trideuterotocainide [2-amino-N-(2,6-xylyl)-3,3,3-trideuterio-propanoic acid amide], pyruvic xylidide (H 170/83), 3-(2,6-xylyl)-5-methylhydantoin (tocainide hydantoin, H 170/85), tocainide heptafluorobutyramide (H 191/12) and the compounds given in Table I, except those designated with a "W" which were from Astra (Worcester, MA, U.S.A.). All amines were available as their hydrochlorides.

The methyl carbamate of dibenzylamine was prepared from equimolar amounts of dibenzylamine and methyl chloroformate in dichloromethane in the presence of sodium carbonate. The solution was washed well with dilute sulphuric acid, dilute sodium hydroxide and water.

All solvents were of p.a. grade from commercial sources.

Methods

   Degradation of perfluoroacyl derivatives of tocainide and its analogues. About 50 nmol of the compound to be studied (Table I) were dissolved in 0.5–2 ml of solvent, usually toluene, containing a suitable amount of the methyl carbamate of dibenzylamine as a marker. The reagent, 2% (0.08 *M*) of heptafluorobutyric anhydride, was then added. The solution was mixed thoroughly and allowed to stand for the times indicated in Figs. 3 and 4. Aliquots were withdrawn and subjected to GC analysis (1–2 μl injected) after a 100-fold dilution in ethyl acetate or as stated otherwise. In some instances the dilution was preceded by evaporation under a stream of nitrogen or by washing with buffer pH 7.4.

   For liquid chromatographic analysis, the reaction solution (1–10 μl) and 50 μl of the mobile phase were injected. The collected fractions were subjected to GC analysis.

   Hydration of degraded HFB-tocainide to give HFB-tocainide. An aliquot of a degradation solution obtained as above was evaporated to dryness in a stream of nitrogen and then dissolved in acetonitrile–water (1:1) or in methanol. After an appropriate time the solvent was changed after evaporation to ethyl acetate and then analyzed by GC with a chiral (for details see ref. 1) or an achiral column.

   Samples for mass spectral analysis. The samples were prepared by reacting 100 μg (0.5 μmol) of the compound with 2% HFBA in toluene. After 30 min at 70°C the solution was analyzed directly (about 1 μg injected).

RESULTS AND DISCUSSION

Heptafluorobutyrylation of tocainide

   Toluene has been used as the solvent for the analytical heptafluorobutyrylation of tocainide with a reagent concentration of about 0.01%. Higher reagent concentrations lead to degradation of the derivative. This is also the case in other solvents. Even with the pure HFB derivative of tocainide severe degradation was observed in different solvents including acetonitrile, diethyl ether, ethyl acetate and dichloro-
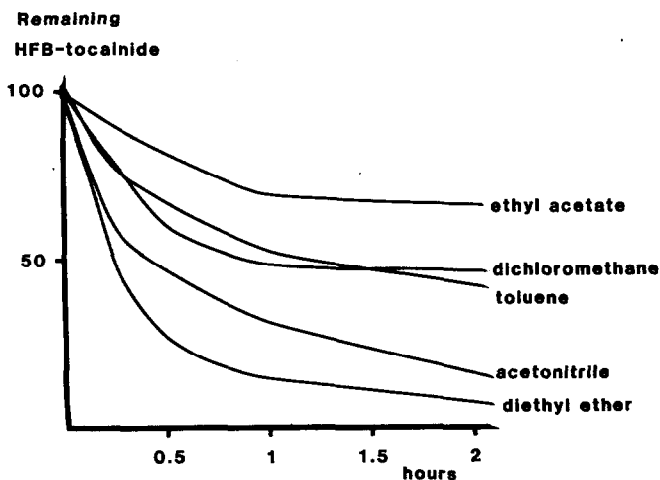

Fig. 3. Time course for the degradation of HFB-tocainide in different solvents with 2% HFBA at 25°C.

methane (Fig. 3). Some degradation was also observed in hexane, however, loss of the derivative during its transfer from the toluene reference solution to hexane was a greater problem.

Stability of perfluoroacylated tocainide

The stability of trifluoroacetyl and pentafluoropropionyl derivatives in the presence of a 0.08 *M* excess of the corresponding anhydrides in toluene was also investigated. The results are given in Fig. 4. The trifluoroacetyl derivative was shown to be more prone to degradation than the HFB derivative, about 50% of the initially formed product disappearing within 30 min. Intermediate behaviour was found for the pentafluoropropionyl derivative.

The degradation of HFB-tocainide in the presence of an excess of anhydride was minimized when the concentration of the anhydride was kept low. An alternative route to the formation of the HFB derivative would be the use of heptafluorobutyryl imidazole[1] and heptafluorobutyryl chloride or N-methyl-bis(heptafluorobutyramide). The same molar concentration (0.08 *M*) of either of the last two reagents resulted in no degradation of HFB-tocainide for up to 2 h at 40°C.

Heptafluorobutyrylation of related compounds

A number of tocainide-related compounds (Table I) were studied in order to see which structural features lead to instability of the derivatives when exposed to an excess of HFBA for at least 2 h at 40°C. Compounds which contain a primary amine and a secondary amide group linked by one or two carbon atoms form unstable derivatives. The substituent of the secondary amide can be aromatic or benzylic. Stable derivatives (less than 5% degradation as compared with time 0) were formed from compounds with a secondary amino group (desethyllidocaine). They can also
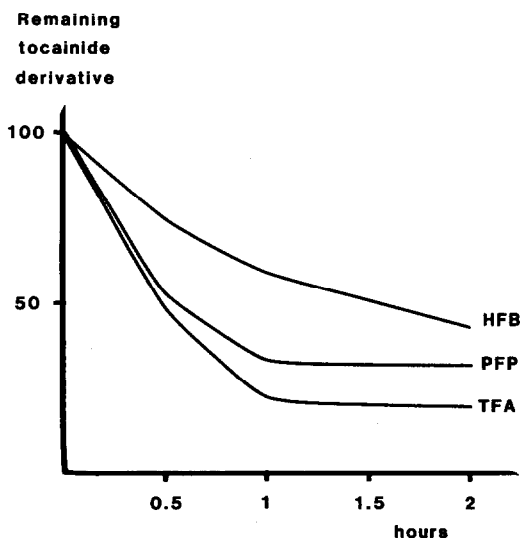

Fig. 4. Time course for the degradation of perfluoroacyl tocainide derivatives obtained with corresponding perfluoroacyl anhydrides (0.08 *M*) at 25°C.

TABLE I

STABILITY OF HFB DERIVATIVES IN TOLUENE AT 40°C WITH 2% HFBA

Parent compound	Structure	Stable HFB* derivative

Tocainide (*cf.*, Fig. 1A)	2,6-Xylyl–NHCO–CH–NH$_2$
	             |
	             CH$_3$
		No

H 132/89	2-Tolyl–NHCO–CH–NH$_2$
	          |
	          CH$_3$
		No

H 151/46	2,6-Xylyl–NHCO–CH–CH$_2$–NH$_2$
	             |
	             CH$_3$
		No

H 155/73	2,4,6-Mesityl–NHCO–CH–NH$_2$
	              |
	              CH$_3$
		No

H 193/10	Phenyl–NHCO–CH–NH$_2$
	          |
	          CH$_3$
		No

H 193/12	Benzyl–NHCO–CH–NH$_2$
	          |
	          CH$_3$
		No

H 195/60	2,6-Xylyl–N(CH$_3$)CO–CH–NH$_2$
	              |
	              CH$_3$
		Yes

N-Desethyllidocaine	2,6-Xylyl-NHCO–CH$_2$–NHC$_2$H$_5$	Yes

W 36149	2,6-Xylyl–NHCO–CH–NH$_2$
	            |
	            C$_2$H$_5$
		No

W 36196	2,6-Xylyl—NHCO–CH$_2$CH–NH$_2$
	              |
	              CH$_3$
		No

W 49167	2,6-Xylyl–NHCO–CH$_2$–NH$_2$	No

Mexiletine	2,6-Xylyl-OCH$_2$–CH–NH$_2$
	            |
	            CH$_3$
		Yes

* The derivatives were considered stable if $\geqslant$ 95% remained after 2 h.

have a methylated aromatic amide nitrogen (H 195/60). Consequently, mexiletine with an oxymethylene bridge to the aromatic ring was stable.

Structure of degradation products

Structural elucidation of the degradation products was achieved by mass spectrometry. A partially degraded solution of HFB-tocainide was analyzed by GC and at least six new peaks were found with shorter retention times than that of the initially formed HFB-tocainide derivative (Fig. 2). Mass spectral analysis revealed that two of these peaks corresponded to the loss of water from HFB-tocainide (c, d), one appeared to be pyruvic xylidide (e), one was devoid of two hydrogens (f) and one had lost both water and two hydrogens (b). The peak (a) with the shortest retention time exhibited the same GC and mass spectrometric (MS) characteristics as the HFB-xylidine reference. The molecular ions were confirmed by chemical ionization mass spectrometry. Degraded HFB-tocainide in solution could be converted into HFB-tocainide (>95%) when the residue after evaporation was dissolved in acetonitrile–water (1:1) or in methanol. The corresponding experiment with $R(-)$tocainide revealed that the chirality of the derivative was lost, as verified by analysis with a Chirasil–Val® column.

Pyruvic xylidide. The presence of pyruvic xylidide (peak e in Fig. 2) was confirmed by comparison of the recorded mass spectrum with that of authentic pyruvic xylidide. (A full mass spectrum of pyruvic xylidide has been published elsewhere[4].) However, it appeared to be formed in the GC system as its retention time differed slightly from that of the authentic sample. This was also the case with "apparent" pyruvic xylidide formed after treatment of tocainide with trifluoroacetyl (TFA) and pentafluoropropionyl (PFP) anhydrides. Corresponding ketoacid xylidides were also observed in the MS analysis of degraded solutions of H 155/73, H 132/89 and H 193/10 (Table I). The term "apparent" pyruvic xylidide is appropriate because this peak can be converted back into HFB-tocainide as discussed above for $R(-)$tocainide. Consequently, the HFB group was still attached to the intermediary degradation product in the solution which was then decomposed in the chromatographic system. However, this compound was observed only after GC on packed columns and not on capillary columns. It is interesting that pyruvic xylidide has also been reported as a metabolite of tocainide in rats[4].

Dehydrated HFB-tocainide. Two peaks corresponding to dehydrated HFB-tocainide were found (c and d, Fig. 2). Their mass spectra are presented in Fig. 5a and b. The general appearance of these spectra are strikingly different, but the molecular ions at 370 a.m.u. exhibited the same elemental composition [370.0878 + 3.7 (= deviation from true value) ppm, $C_{15}H_{13}ON_2F_7$, peak d; 370.0866 + 4.9 ppm, $C_{15}H_{13}ON_2F_7$ peak c] indicating isomeric structures. Compared to the mass spectrum of HFB-tocainide[2], the mass spectrum of the major compound (peak c) with shorter retention time indicated that the amide was now a tertiary one as the ion at m/z 147 (147.0688 C_9H_9ON + 0.5 ppm) was prominent instead of that at m/z 148 for HFB-tocainide. A similar change has been observed in the mass spectrum of tocainide hydantoin[4], also a tertiary amide. The perfluoroacyl group now appeared to be aliphatic as M − 119 (C_2F_5) at m/z 251 (251.0722 + 3.4 ppm $C_{13}H_{13}ON_2F_2$) was prominent. The resonance stabilization of this ion is likely to be a driving force for its formation. This ion was shifted to m/z 254 in the spectrum of
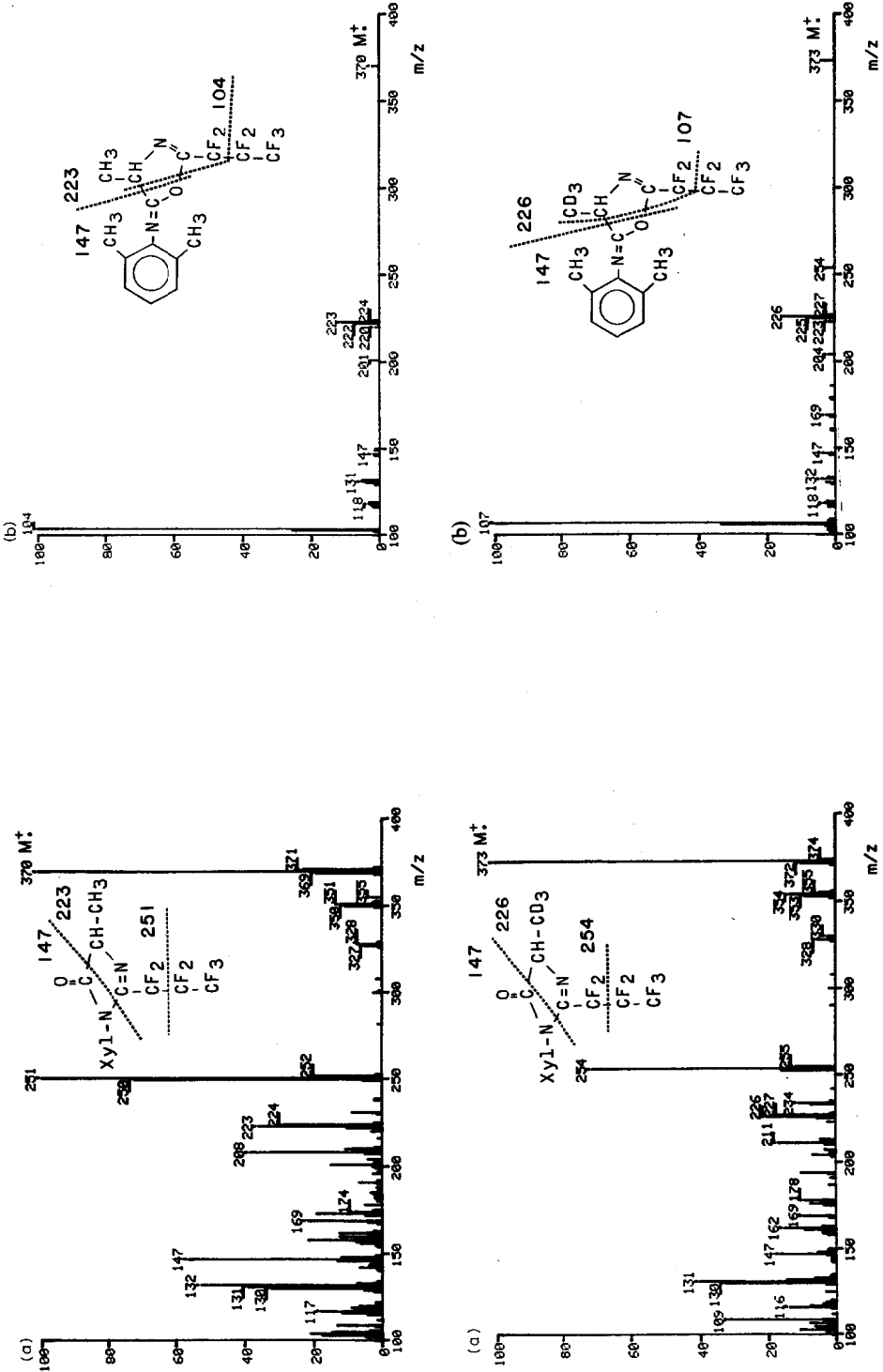
Fig. 5. Electron-impact mass spectra (70 eV) of dehydrated HFB-tocainide (upper part): (a) peak d and (b) peak c in Fig. 2. Corresponding spectra from degraded deuterotocainide are given in the lower part.

the corresponding trideuterotocainide, confirming the loss of the C_2F_5 group from the molecular ion. Fluorine expulsion from the molecular ion was also observed (m/z 351). The spectrum of the minor dehydration product from HFB-tocainide showed less fragmentation (Fig. 5b). The molecular ion was present (see above) together with a peak at m/z 223 (223.0243 − 1.3 ppm $C_6H_4NF_7$). Assuming the loss of 147 a.m.u. from the molecular ion, this would also suggest a cyclic structure as discussed above. The base peak at m/z 104 is thought to emanate from the m/z 223 ion by the elimination of the perfluoroalkyl fragment C_2F_5. This ion was shifted by 3 a.m.u. in the spectrum of trideuterotocainide, consistent with the proposed fragment ion given in Fig. 5b. The suggested structure for this ion is similar to one formed by electron impact fragmentation of oxazolines[5] formed from N-acetylnorephedrine as a side-product.

The formation of a cyclic derivative from HFB-tocainide, a diamide, resembles the last step in the synthesis of methaqualone[6]. Other somewhat related reactions are the formation of a spiro cyclic derivative from melatonin by the action of pentafluoropropionic anhydride[7], the formation of oxazolidinones from amino acids and trifluoroacetic anhydride[8] and the formation of oxazolidines from perfluoroacetylated N-acetylnorephedrine compounds[5]. The dehydrating properties of perfluoro anhydrides have been used in the GC determination of compounds containing primary amide groups as their nitrile derivatives[9]. The tocainide hydantoin[4,10] is evidence of the tendency of tocainide and its derivatives to form cyclic structures.

Dehydrogenated HFB-tocainide. A peak with GC retention similar to that of HFB-tocainide (Fig. 2f) had the mass spectrum shown in Fig. 6. From the molecular ion (386.0853 + 1.0 ppm $C_{15}H_{13}O_2N_2F_7$) one can conclude that two hydrogens have been lost from HFB-tocainide by oxidation. The presence of an ion at m/z 238 (238.0124 − 2.3 ppm $C_6H_3ONF_7$) and not m/z 240 indicated that a double bond was formed in the perfluoroacyl part of the molecule and not near the aromatic ring. Evidence in support of this assumption was provided by the presence of the ion at m/z 148 (148.0740 + 2.1 ppm $C_9H_{10}ON$). The mass spectra of deuteromethyl HFB-tocainide indicated that one deuterium has been lost by oxidation. This may be explained by the presence of a mesomeric structure in which the double bond involves both the methyl group and the perfluoroacylamide nitrogen. The base peak at m/z 121 (121.0905 − 1.4 ppm $C_8H_{11}N$) is consistent with the base peak in the spectrum of HFB-tocainide[2]. Dehydrogenation was also observed with the compounds H 155/73, H 193/10 and W 36149. In the last case the retention time was longer than that of the parent compound.

The oxidative properties of perfluoro anhydrides have been observed with triethylamine and trifluoroacetic anhydride[11]. The reactions observed here also bear some resemblance to the perfluoroacylation of desipramine[12] and imipramine[13] and also to those of noscapine and morphine derivatives[14]. In these cases vinylogous HFB amides are formed.

Dehydrogenated and dehydrated HFB-tocainide. Upon prolonged degradation of HFB-tocainide, at elevated temperature and in the presence of an excess of anhydride, peak b (Fig. 2) became the main component in the chromatogram and accounted for 80% of the products detected. Attempts to use other spectroscopic techniques were hampered by the instability of this compound, especially in the presence of moisture. Evidence for the position of the double bond was obtained by the
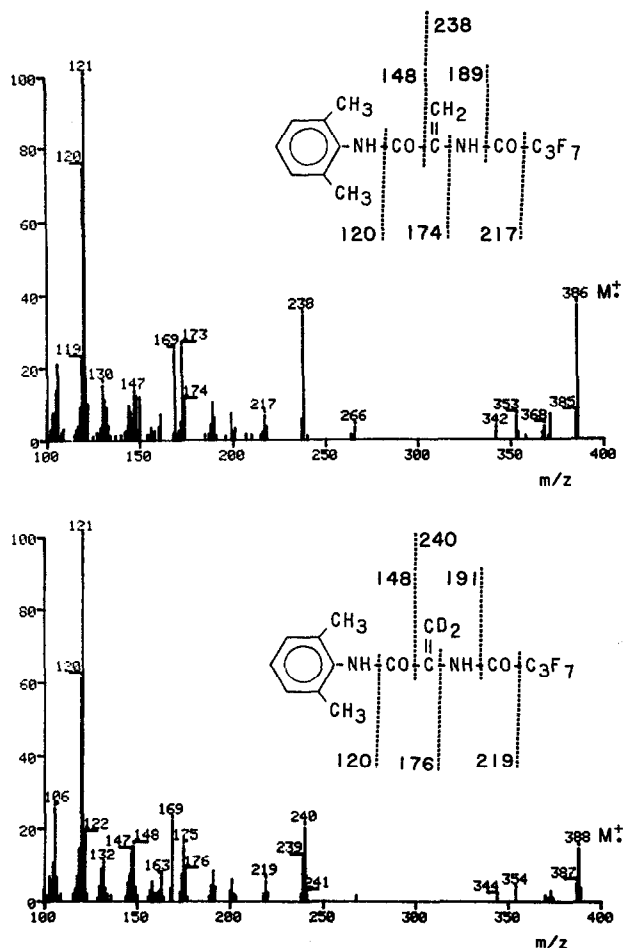
Fig. 6. Electron impact mass spectra (70 eV) of dehydrogenated HFB-tocainide, peak f in Fig. 2 and corresponding spectra from HFB-deuterotocainide (lower part).

mass spectrum of degraded deuteromethyl HFB-tocainide and of the compound H 155/73 (Fig. 7). Observed key ions included 199 (199.0840 + 3.0 ppm $C_{12}H_{11}ON_2$), 156 (156.0832 − 2.0 ppm $C_{11}H_{10}N$) and 130 (129.9972 + 0.5 ppm $C_4ON_2F_2$). The ion at m/z 199 was apparently formed by complete elimination of the perfluoroalkyl side chain (169). Then HCNO (43) was eliminated and a peak at m/z 156 was formed. In the case of trideuterotocainide and H 155/73, ions were observed at m/z 201–158 and 213–170, respectively, supporting the fragmentation pathway discussed above. When a reaction solution was monitored after evaporation, and not directly after dilution of an aliquot, the dehydrated and dehydrogenated HFB-tocainide, peak b (Fig. 2), was overestimated compared to the major dehydration peak (c, Fig. 2). The latter could be increased by treatment of the solution with heptafluorobutyric acid (1%) which caused a simultaneous decrease in peak b (Fig. 2). Attempts failed specifically to reduce the double bond in the dehydrated and dehydrogenated HFB-
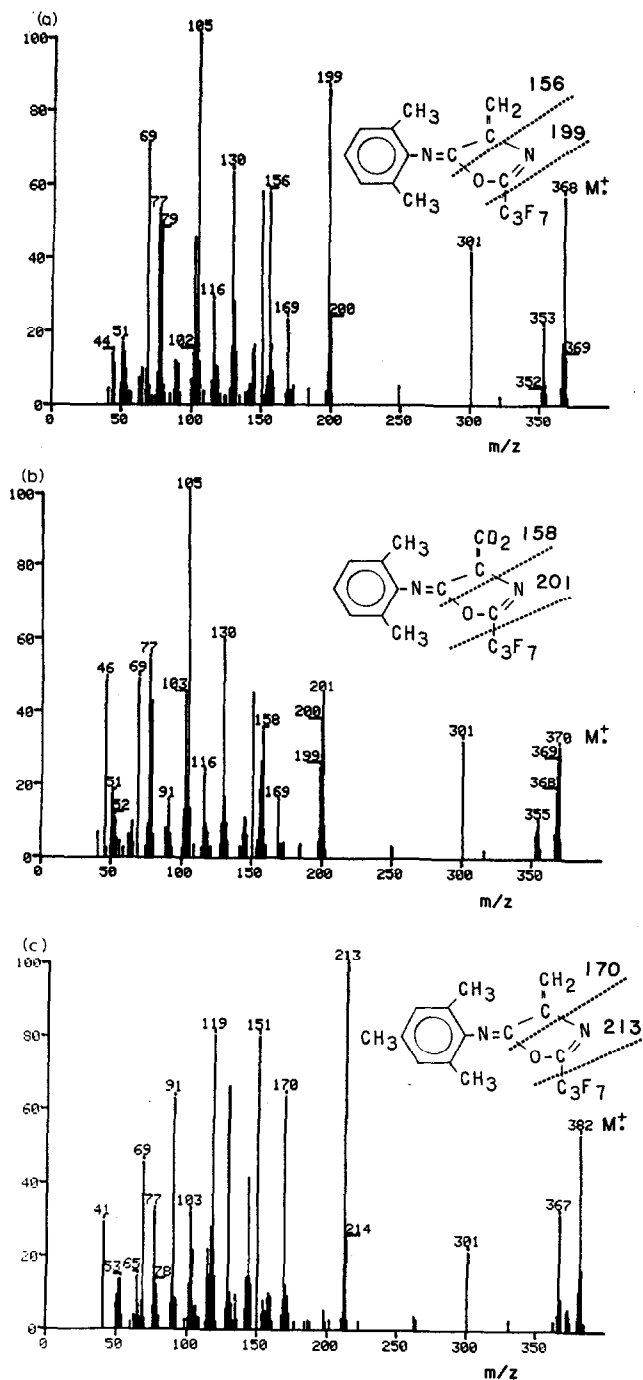
Fig. 7. Electron-impact mass spectra (70 eV) of dehydrated and dehydrogenated HFB-tocainide (a), from the corresponding HFB-deuterotocainide peak (b) and from the corresponding HFB-H 155/73 peak (c).

tocainide compound with NaBH$_4$. In this experiment, HFB-tocainide was formed together with minor amounts of HFB-xylidine when the solvent was toluene. A minor amount of dehydrated HFB-tocainide was also observed in hexane as solvent. Similar reactions have been reported in the synthesis of methaqualone metabolites with lithium borohydride[6].

   HFB-xylidine. Peak a (Fig. 2) was identified as HFB-xylidine from its mass spectrum (M$^+$, m/z 317) and by comparison with the chromatographic retention time of authentic HFB-xylidine. The reference was prepared by reacting xylidine with HFBA. In some instances, bis(heptafluorobutyryl)xylidine was observed with a shorter retention time. The molecular ion was not recorded, but the presence of an ion at m/z 344 (M $-$ C$_3$F$_7$) followed by one at m/z 316 (M $-$ COC$_3$F$_7$) and the shorter retention times support this assumption. The formation of bis(perfluoroacyl) derivatives of amines has been reported elsewhere[15].

Liquid chromatographic studies
   Solutions of degraded HFB-tocainide were subjected to liquid chromatographic analysis with two different mobile phase systems and LiChrosorb Si 60 as support.
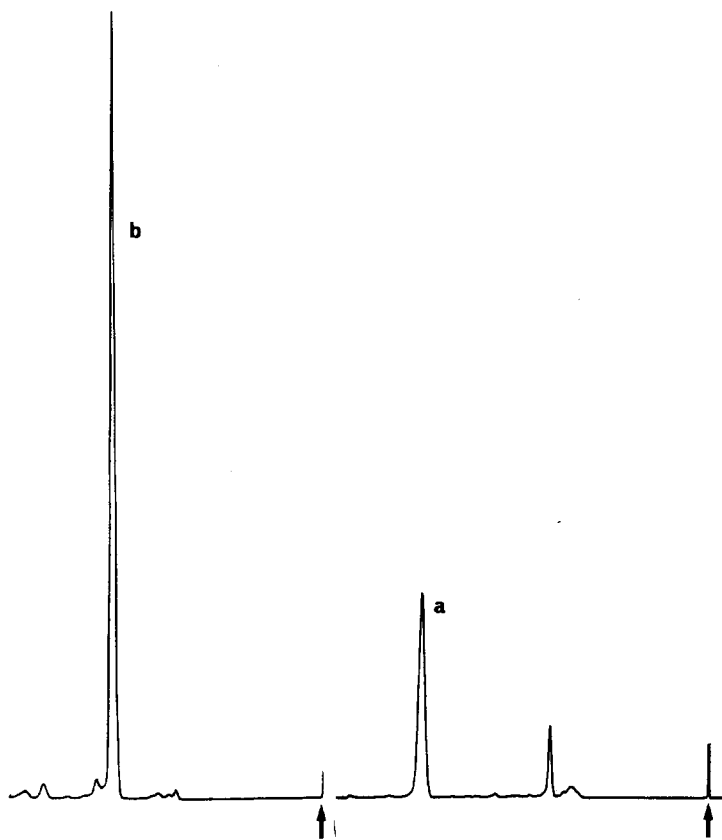
Fig. 8. Liquid chromatograms of HFB-tocainide (a) and a degraded solution of HFB-tocainide (b) using system A and a 5-μm LiChrosorb Si 60 column. UV detection at 254 nm.

TABLE II

LIQUID CHROMATOGRAPHIC RETENTION, k'

Two chromatograms with system A are given in Fig. 8.

Compound	Mobile phase	
	5% Propanol/hexane, 0.1% acetic acid, 0.1% water (system A) (t_0 = 2.0 min)	10% Tetrahydrofuran/hexane (system B) (t_0 = 2.1 min)
HFB-tocainide	0.8	5.2
Dehydrated and dehydrogenated HFB-tocainide	0.25	0.88
HFB-xylidine	n.m.*	0.52
Pyruvic xylidide	n.m.	1.28

* n.m. = Not measured.

The results are summarized in Table II and chromatograms are given in Fig. 8.

Peaks collected using system A were analyzed by GC to confirm their identity by retention data. Gas chromatography of the fraction containing the main peak (Fig. 8, b) after degradation of HFB-tocainide (Fig. 8, a) gave upon GC dehydrated and dehydrogenated HFB-tocainide as the major peak and a trace of HFB-tocainide, probably formed by hydration. The compound showed signs of degradation in the GC system, e.g., an elevated baseline for about 1 min after the main peak had eluted. The baseline elevation could be eliminated by increasing the injector temperature from 250 to 300°C. These results indicate that the compound collected by liquid chromatography was not identical with that detected by GC. Furthermore, direct inlet MS of the liquid chromatographic peak gave a molecular ion at m/z 370 as opposed to one at m/z 368 obtained after GC.
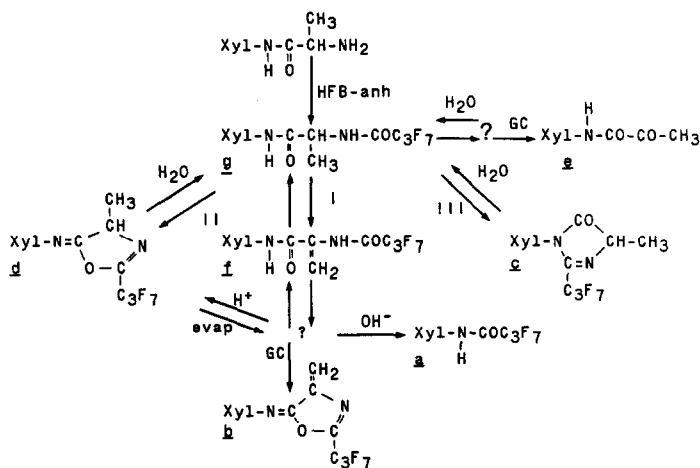

Fig. 9. Proposed routes for the degradation of HFB-tocainide. The letters refer to the peaks in the chromatogram (Fig. 2). I, Oxidation route; II, cyclization with C=O as nucleophile and III, cyclization with NH as nucleophile.

The reaction of HFB-tocainide to mainly dehydrated and dehydrogenated HFB-tocainide resulted in an increase in absorbance at 254 nm by about a factor of 5.

The liquid chromatographic experiments demonstrate that the degradation of HFB-tocainide takes place during the derivatization and is not a mere artifact of GC.

CONCLUSIONS

HFB-tocainide undergoes a series of reactions in the presence of an excess of anhydride and is transformed to several products; these reactions can be reversed upon contact with water. However, the degradation of HFB-tocainide in the presence of an excess of anhydride can be minimized if the concentration of the anhydride is kept low[1]. An alternative route to the derivative formation can also be employed such as reaction with heptafluorobutyryl chloride or N-methyl-bis(heptafluorobutyramide). Structures for the degradation products formed were proposed mainly on the basis of MS analysis. No total synthesis of the suggested structures was attempted as they are relatively unstable and sensitive to moisture and acid. The proposed structures are thus only tentative. A scheme for the reactions reported is given in Fig. 9.

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