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Electron Impact Fragmentation Studies of β-Blocking Drugs and Their Metabolites by GC–Mass Spectroscopy

DANIEL A. GARTEIZ▲ and THOMAS WALLE

Abstract □ This work describes the mass fragmentation patterns of trifluoroacetylated derivatives of five *aryloxy* β-blocking drugs as well as five metabolites of this chemical and therapeutic class. This chemical class is characterized by intense ions at *m/e* 308, 266, and 43 and by a strong metastable ion at *m/e* 229.2. The mechanism of fragmentation was confirmed with the hexadeuterated (d_6) analogs of these compounds. In addition, mass spectral features are described for three *arylalkyl* β-blocking drugs. These mass spectral data should facilitate the rapid and accurate determination of the metabolic fate of these and other β-blocking drugs of this chemical class.

Keyphrases □ β-Adrenergic blocking agents and metabolites—mass fragmentation patterns of trifluoroacetyl derivatives □ Metabolites of β-adrenergic blocking agents—mass fragmentation patterns of trifluoroacetyl derivatives □ Electron impact fragmentation patterns—β-adrenergic blocking agents and metabolites, GC–mass spectroscopy □ GC–mass spectroscopy—electron impact fragmentation patterns, β-adrenergic blocking agents and metabolites

Rigorous qualitative or quantitative studies in drug metabolism ultimately require proof of the molecular structures under investigation. These structure determinations must frequently be carried out on submicrogram quantities of drugs and drug metabolites in complex chemical mixtures of biological origin and can become difficult, time consuming, and expensive.

The combined gas chromatograph–mass spectrometer has provided a powerful tool to facilitate structure elucidation of compounds eluting from a GC column. Proof of structure by this technique still requires a comparison of recorded mass spectra to those of pure reference compounds, or a thorough knowledge of rigorously established fragmentation mechanisms of the chemical class under investigation may suffice.

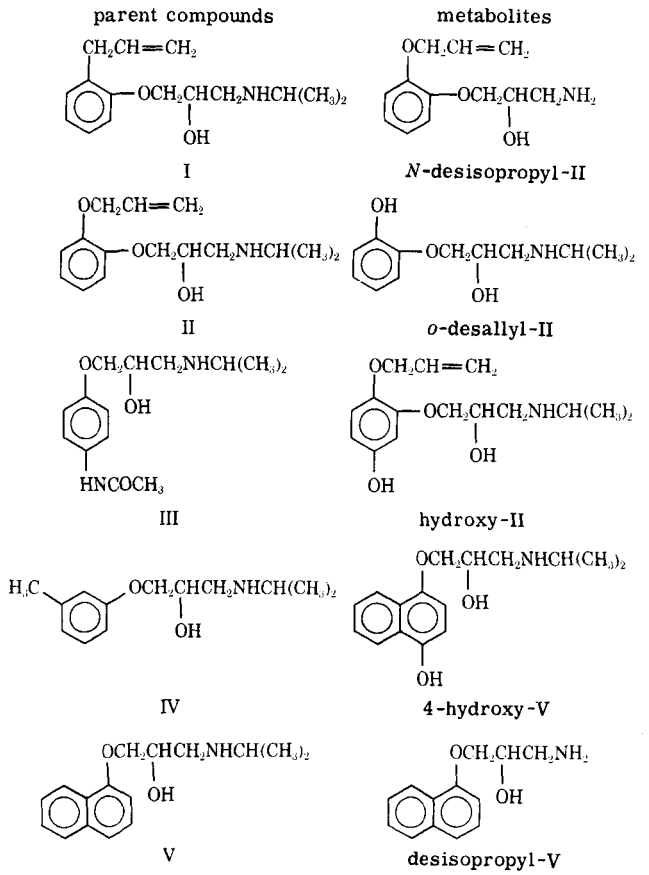
This report describes electron impact fragmentation patterns and associated mechanisms for trifluoroacetylated derivatives of a pharmacologically complex and

increasingly prescribed therapeutic and chemical class of drugs, the β-adrenergic receptor blocking agents.

EXPERIMENTAL

Materials and Methods—Currently available compounds can be subdivided into the *aryloxy* β-blockers, which contain a β-hydroxy-*N*-isopropylaminopropylene side chain connected to a ring system through an ether linkage, and *arylalkyl* β-blockers, which contain a β-hydroxy-*N*-isopropylaminoethylene side chain directly attached to the ring system.

The chemical structures of the drugs and metabolites studied are given in the text and Table I. Alprenolol (I), oxprenolol (II), sotalol,



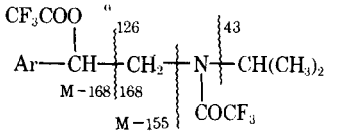
Structural formulas for aryloxy β-blocking drugs and their desalkyl and hydroxy metabolites

and the *N*-desisopropyl metabolite of oxprenolol (*N*-desisopropyl-II) were used[1]. The dichloro derivative of isoproterenol (VI), practolol (III), 1-isopropylamino-3-(*m*-tolyloxy)-2-propanol[2] (IV), pronethalol, propranolol (V), and the 4-hydroxy metabolite of propranolol (4-hydroxy-V) were obtained from a commercial source[3]. The *N*-desisopropyl metabolite of propranolol (*N*-desisopropyl-V) was synthesized as described earlier (1).

The hexadeuterated (d_6) materials, 1-(d_6-isopropylamino)-3-(1-naphthyloxy)-2-propanol (propranolol-d_6) and 1-[*o*-(allyloxy)phenoxy]-3-(d_6-isopropylamino)-2-propanol (oxprenolol-d_6), were prepared from 1-amino-3-(1-naphthyloxy)-2-propanol (*N*-desisopropyl-V) and 1-[*o*-(allyloxy)phenoxy]-3-amino-2-propanol (*N*-desisopropyl-II), respectively, through Schiff-base formation with acetone-d_6 and subsequent reduction with sodium borohydride (2).

[1] Obtained from William S. Merrell Co.
[2] ICI-45763.
[3] Imperial Chemical Industries.

Table I—Mass Spectral Data of Di(trifluoroacetyl) Arylalkyl β-Blocking Drugs



m/e	Relative Intensity, %: Pronethalol Ar = β-Naphthyl	Sotalol Ar = p-CH3SO2NH-phenyl	VI Ar = 3,4-Dichlorophenyl
126	100	100	100
168	60	100	70
43	51	71	90
M − 155	69	18	7
M − 168	23	2	5
M − CF3COOH	10	12	2
M	10	0.1	0.1

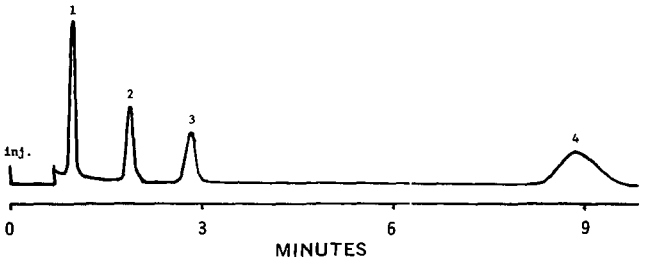


Figure 1—*Total ion current recording of di(trifluoroacetyl) aryloxy β-blocking drugs. Key: 1, 1-isopropylamino-3-(m-tolyloxy)-2-propanol; 2, alprenolol; 3, oxprenolol; and 4, propranolol.*

The *o*-desallyl metabolite of oxprenolol (*o*-desallyl-II), the ring-hydroxy metabolite of oxprenolol (hydroxy-II), and their d_6-labeled analogs were isolated and structurally characterized by GC–mass spectroscopy from rat urine extracts. Rats were given a 10-mg./kg. dose of oxprenolol or d_6-labeled oxprenolol intraperitoneally and their urine was collected for 24 hr.

Isolation and Derivatization Procedures—Reference compounds were derivatized in benzene with trifluoroacetic anhydride in the presence of a small amount of trimethylamine. The reaction mixtures were heated at 50° for 5 min. and shaken with distilled water prior to injection into the gas chromatograph–mass spectrometer.

Isolation procedures from biological material of parent drug and metabolites were described previously for oxprenolol (3) and propranolol (1).

GC–Mass Spectroscopy—The combination instrument[4] was used at an accelerating voltage of 3.5 kv. and an ionization voltage of 20 ev., unless otherwise stated, and a trap current of 65 μamp. A 90-cm. × 2-mm. Pyrex glass column was used containing 1% OV-17 on Chromosorb W, 80–100, AW-DMCS, HP[5]. Injector temperature was 250°, column temperature was 150–200°, and separator temperature was 280°. The carrier gas flow rate was 10 ml./min.

In all mass spectra shown, fragment ions with intensities less than 5% of the base peaks as well as all isotope peaks have been excluded for simplicity.

RESULTS AND DISCUSSION

Aryloxy β-Blocking Drugs—The volatility of the trifluoroacetylated aryloxy β-blockers is strongly enhanced compared to the underivatized compounds, as is the peak symmetry (Fig. 1). The mass spectra of trifluoroacetylated aryloxy β-blockers are shown in Fig. 2.

[4] LKB 9000.
[5] Varian.

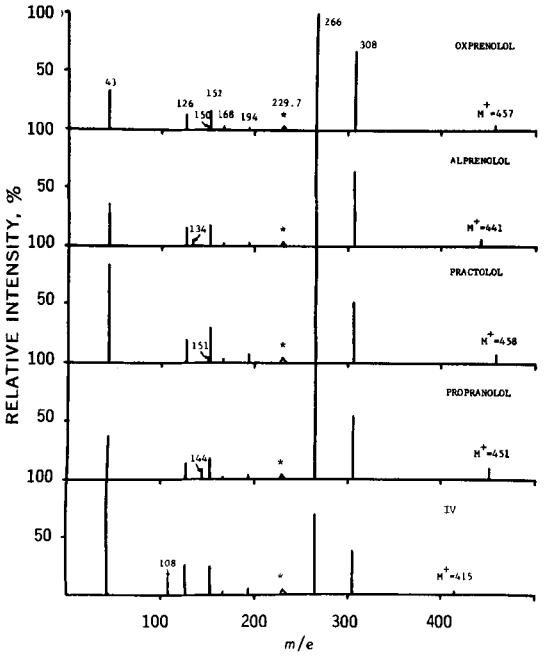


Figure 2—*Mass spectra of di(trifluoroacetyl) aryloxy β-blocking drugs (70 ev.).*

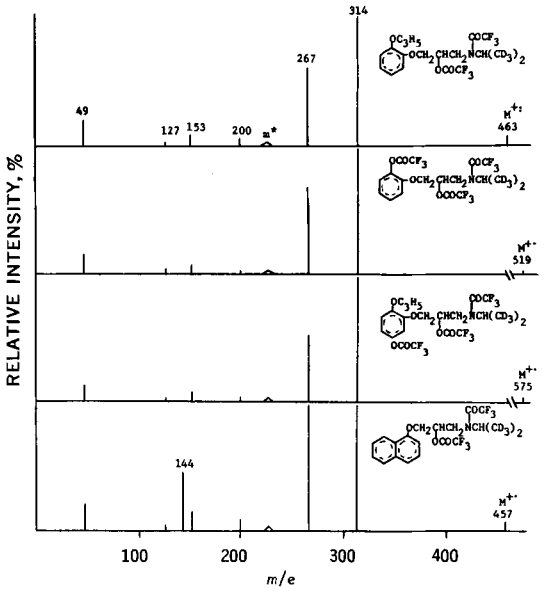
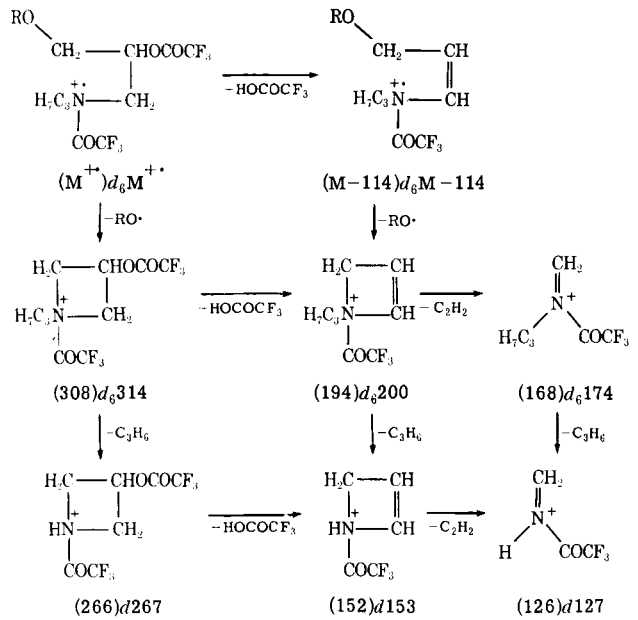


Figure 3—*Mass spectra of di(trifluoroacetyl) hexadeuterated aryloxy β-blocking drugs.*

The molecular ions for each compound are consistent with formation of a di(trifluoroacetyl) derivative. The striking similarity among the mass spectra results from abundant (*m/e* 308, 266, and 43) fragment ions and an intense metastable ion (m* 229.2). Other common fragment ions are found at *m/e* 194, 168, 152, and 126.

The proposed mechanism giving rise to the observed fragmentation pattern of these trifluoroacetylated derivatives is shown in Scheme I. The molecular ions undergo primary fragmentation through cleavage on the alkyl side to the ether linkage, giving rise to *m/e* 308, the intact side chain as the base peak at 20 ev. This ion (*m/e* 308) undergoes further fragmentation with loss of C_3H_6 to yield another abundant ion (*m/e* 266). The transition 308 → 266 involves a "McLafferty-type" rearrangement of a hydrogen of one of the methyls of the isopropyl group to the nitrogen atom. This was



Scheme I—Mechanistic pathways for the observed fragmentation patterns of trifluoroacetylated derivatives of aryloxy β-blocking drugs. Fragment ions are shown for the unlabeled and deuterium (d)*-labeled compounds.*

confirmed with the d_6-labeled compounds. An intense metastable ion (m* 229.2) is observed for this transition and appears as a broad diffuse peak in all the mass spectra. The two most intense fragment ions (*m/e* 308 and 266), as well as the molecular ions, undergo loss of trifluoroacetic acid, giving rise to *m/e* 194 and 152 and M − 114, respectively. Both *m/e* 194 and 152 undergo loss of C_2H_2, yielding *m/e* 168 and 126, respectively. The fragment ions (*m/e* 152 and 126) may also arise from *m/e* 194 and 168 through loss of C_3H_6, as occurs with the base peak. However, metastable peaks for these transitions were not observed.

The fragmentation mechanism shown in Scheme I was confirmed by the mass spectra of propranolol-d_6 and oxprenolol-d_6 and oxprenolol-d_6 metabolites (Fig. 3). The molecular ions of all the hexadeuterated molecules were found 6 atomic mass units (a.m.u.) higher than for the respective undeuterated compounds. Furthermore, the fragment ions (*m/e* 308, 194, and 168) of the unlabeled molecules proposed to contain the isopropyl group also appeared 6 a.m.u. higher (*m/e* 314, 200, and 174) for the d_6-labeled molecules (Scheme I and Fig. 3). In addition, *m/e* 266 of the unlabeled molecules was found 1 a.m.u. higher for the d_6-labeled compounds. This confirms that the transition 308 → 266 involves a McLafferty-type rearrangement of a hydrogen of one of the methyls of the isopropyl group to the nitrogen atom. Furthermore, the metastable peak for this transition was found to shift to 227.0, which corresponds to the predicted ratio $(267)^2/314$. The fragment ions *m/e* 152 and 126 of the unlabeled molecules, as expected, were found 1 a.m.u. higher in the mass spectra of the d_6-labeled compound.

Mass spectra recorded at 70 ev. showed only minor intensity changes when compared to those recorded at 20 ev. For the *aryloxy* β-blockers, a reversal in the intensities of masses 308 and 266 was observed, *m/e* 308 being the base peak at 20 ev. and *m/e* 266 the base peak at 70 ev. For all compounds, more intense molecular ions were observed at the lower voltage.

N-Desalkyl Metabolites of Aryloxy β-Blocking Drugs—The fragmentation pattern of the trifluoroacetylated desisopropyl metabolites of oxprenolol and propranolol is similar to that of the parent drugs (Fig. 4). Fragment ions *m/e* 266, 152, and 126 and M − 114 are all present in the mass spectra. Fragment ions *m/e* 308, 194, 168, and m* 229.2 are not present since these molecules do not contain the isopropyl group.

The base peak for trifluoroacetylated desisopropyl-V is *m/e* 144, and the base peak for trifluoroacetylated desisopropyl-II is *m/e* 150. These fragment ions presumably arise through abstraction of a side-chain hydrogen by the oxygen during cleavage of the ether linkage, as shown in Scheme II. Nonspecific hydrogen rearrangements of this type were described previously for phenyl alkyl ethers (4). Fragment ions arising through abstraction of side-chain hydrogens are also present in the mass spectra of all the parent aryloxy β-blocking

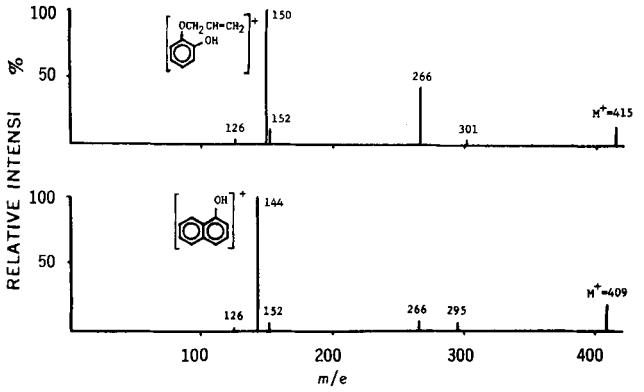
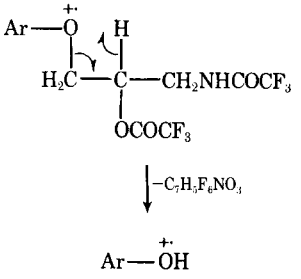


Figure 4—*Mass spectra of the di(trifluoroacetyl)* **N***-desisopropyl metabolites of oxprenolol (top) and propranolol (bottom).*



Scheme II

drugs (*m/e* 150, 134, 151, 144, 108) but are of low abundance (Fig. 2).

Arylalkyl β-Blocking Drugs—Mass spectra of the trifluoroacetylated derivatives of pronethalol, sotalol, and VI were also recorded. The major mass spectral features characterizing these compounds (Table I) arise from the double α- and C—N cleavage, with hydrogen rearrangement typical of secondary and tertiary amides (5). In addition, the molecular ions undergo loss of trifluoroacetic acid as was observed for the aryloxy β-blockers. Similar fragmentation patterns have been observed for fluoroacylated catecholamine metabolites (6).

CONCLUSION

This work has described the mass fragmentation patterns of trifluoroacetylated derivatives of aryloxy and arylalkyl β-blocking drugs. For the aryloxy compounds, the fragmentation patterns were confirmed with hexadeuterated molecules. In addition, the mass spectral characteristics of five metabolites of this chemical and therapeutic class have been described.

These mass spectral data will permit rapid structure elucidation of other β-blocking drugs and their metabolites. Furthermore, the excellent GC properties of the trifluoroacetylated derivatives, together with the very intense ions produced upon electron impact, will permit measurements of picogram amounts of these compounds by mass fragmentography (7).

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