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CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC STUDY OF THE EFFECT OF SOLVENTS ON METOPROLOL AND OTHER ARYLOXYPROPANOLAMINES

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SUMMARY

When metoprolol in methanol was analysed by capillary gas chromatography (GC), an additional peak was observed; mass spectrometry (MS) showed this additional peak to have a molecular weight 12 dalton higher than that of the parent compound. A similar phenomenon was observed with other β-adrenergic blocking aryloxypropanolamines in methanol or dichloromethane. Capillary GC–MS using deuterated solvents as isotopic markers showed that a methylene group from the solvents was incorporated into the parent molecule. The structure of the observed products and the mechanism of their formation are proposed.

INTRODUCTION

Metoprolol and other β-adrenergic blockers are widely used in the treatment of hypertension[1]. The structural formulae of the growing group of the beta-adrenergic blocking agents have similar side-chains as may be seen in Table I.

Because of their wide use, various methods have been described for the determination of these β-adrenergic blockers. These methods include high-performance liquid chromatography using fluorometric detection[2], UV-absorption detection[3–5] and electrochemical detection[6], gas–liquid chromatography (GLC) after appropriate derivatization[7–11] and GLC combined with mass spectrometry (MS)[12–14]. When using a capillary gas chromatography (GC)–MS system for the analysis of metoprolol in methanol, we have observed an extra peak which eluted shortly after the metoprolol. This extra peak was not normally seen when a packed column was used. On investigation, a similar phenomenon was observed with other β-adrenergic blocking aryloxypropanolamines in methanol solution. The molecular weight of these extra peaks were found to be 12 dalton higher than that of the corresponding parent compounds. The mass spectra of these β-blockers in methanol solution obtained from capillary GC–MS were compared with the corresponding mass spectra obtained from directly inserted solid samples. The pure spectra of the β-blockers were obtained only by direct insertion. A rearrangement of the molecules of these compounds in the GC

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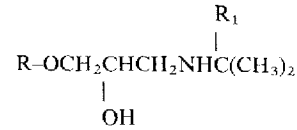
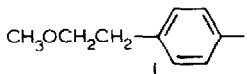
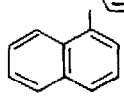
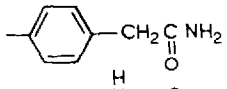
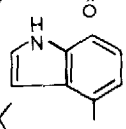
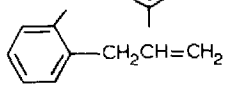
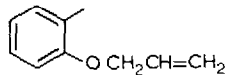
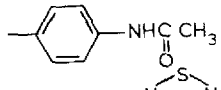
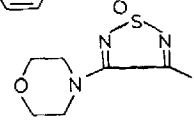
TABLE I

β-ADRENERGIC BLOCKERS STUDIED



Compound	*R*	R_1	*Molecular weight*	
			Parent compound	*GC product*
Metoprolol		H	267	279
Propanolol		H	259	271
Atenolol		H	266	278
Pindolol		H	248	260
Alprenolol		H	249	261
Oxprenolol		H	265	277
Practolol		H	266	278
Timolol		CH_3	316	328

system in the presence of methanol was suspected. In this paper the structure of the GC product and the mechanism of its formation are proposed.

EXPERIMENTAL

Capillary GC–MS system

A Hewlett-Packard 5985A GC–MS system with electron impact (EI) and chemical ionization (CI) capabilities was used. The system was modified so that the fused silica capillary column (FSCC) was threated directly to the ion source, within 2 cm of the electron beam. No effect was observed on the quality of CI spectra obtained due to the presence of 2 ml helium through the FSCC. An all-glass splitless insert (without any glass wool) was used.

Operating conditions

A 12.5 m × 0.22 mm I.D. Hewlett-Packard Ultra 1 (OV-101) bonded-phase FSCC was used. At 10 p.s.i. inlet pressure using helium as the carrier gas, the source pressure was $6 \cdot 10^{-6}$ Torr when the column was at ambient temperature. No difficulty with background was experienced at this pressure.

The sensitivity of the mass spectrometer was normally set at the "Autotune" value for the electron multiplier voltage in the EI mode. For CI mode, 15 ml of pure methane gas was bled in via a separate inlet into the mass spectrometer. The final source pressure was $4 \cdot 10^{-4}$ Torr and no alteration to the retention time of the eluting peaks through the column was observed. GC conditions were: 100°C for 1 min, programmed at 16°C/min to 280°C and then held for 10 min. The injection port temperature was 250°C and the transferred line 270°C. Injections (1 μl) of all samples and standards were made in the splitless mode with a loading time of 0.8 min.

Reagents and materials

All the standards were procurred from the appropriate pharmaceutical company. The compounds investigated were listed in Table I. The following analytical grade solvents, from May & Baker, were used: methanol, toluene, acetone, ethyl acetate, ethanol, acetonitrile, isopropyl alcohol, chloroform and dichloromethane.

Procedures

β-Blockers studied. The β-blockers listed in Table I were studied. Solutions of 100 μg/ml (100 ppm) of these compounds in methanol were prepared and 1 μl of each of these standard solutions were injected using the splitless mode, under both EI and CI conditions. For metoprolol, the standard solutions were prepared using both the free base and its tartrate salt.

Solvent effect. Metoprolol solutions of 100 μg/ml (100 ppm) in solvents mentioned above were prepared and 1 μl was injected using the splitless mode, under both EI and CI conditions.

Deuterated solvents. Metoprolol solutions of 100 ppm in methyl-d_3 alcohol-d ($C^2H_3O^2H$) and dichloromethane-d_2 ($C^2H_2Cl_2$) were prepared.Of each of these solutions 1 μl was injected using the splitless mode, under both EI and CI conditions. The syringe used was rinsed thoroughly with toluene in between each injection to eliminate any possible contamination by methanol.

RESULTS AND DISCUSSION

β-Blockers studied

All the β-blockers studied showed an additional peak which was eluted about 0.5 min later than that of the corresponding parent compounds, a difference corresponding to about 50–70 Kováts indices. This is illustrated in Fig. 1 using metoprolol as an example. The EI mass spectrum of the second peak is shown in Fig. 2a. When using a 1.8 m × 4 mm I.D. 3% OV 101 glass column, this second peak was not well resolved from the parent peak, and the two peaks might even appear as a single peak if the ratio between two peaks was high. The second peak appeared to be a product formed during GC as no similar compound was observed under direct insertion probe conditions. Ubder CI conditions, the molecular weight of the second peak (Fig. 2b)

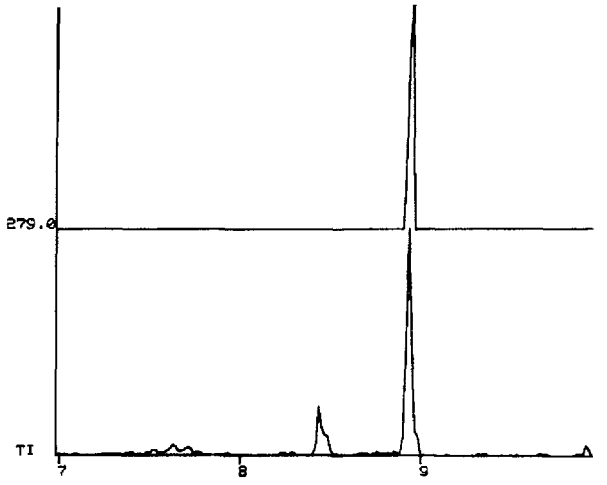


Fig. 1. Total ion chromatogram of metoprolol in methanol.

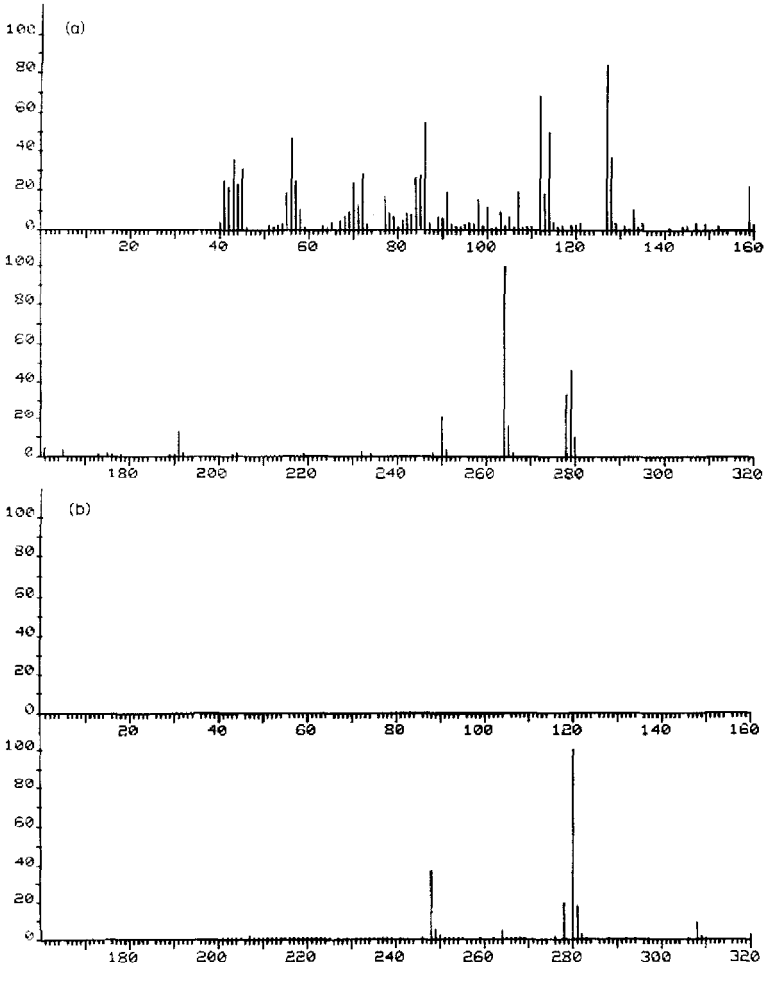


Fig. 2. (a) Metoprolol in methanol, EI mass spectrum of GC product. (b) Metoprolol in methanol, CI mass spectrum of GC product.

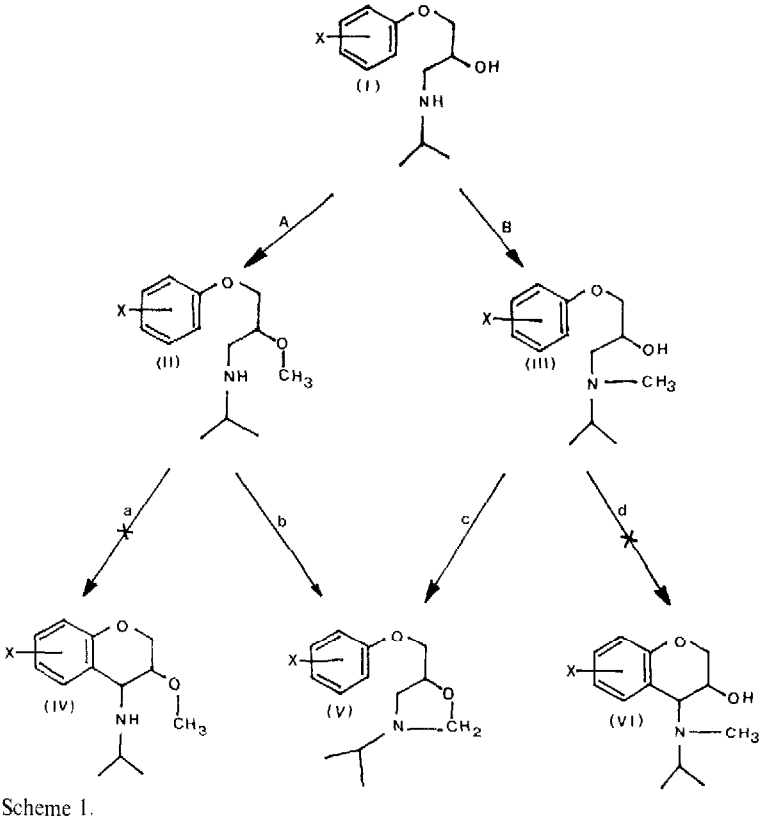
was 12 dalton greater than that of the parent drug metoprolol. There was no difference observed between those standards prepared in its free base or tartrate salt form. Similar GC products were observed with other β-adrenergic blockers as shown in Table I. Such phenomenon was less pronounced when injection port temperature was lowered to 200°C. This indicated the formation of the GC product was temperature related.

Solvent effect

The GC product from metoprolol was formed only with methanol and dichloromethane as solvents. All the other solvents tested *viz.*, toluene, ethyl acetate, acetonitrile, acetone, isopropyl alcohol, chloroform and ethanol did not give the corresponding metoprolol GC product. This indicates that the solvents methanol and dichloromethane are involved in the reaction leading to the formation of the GC product.

Deuterated solvent

With $C^2H_3O^2H$ and $C^2H_2Cl_2$ the molecular weight of the GC products formed were 2 dalton higher than those of the corresponding GC products with methanol and dichloromethane. This information is important to the elucidation of the likely mechanism for the formation and the structure of the GC products as discussed in the next section.



Scheme 1.

Proposed structure of GC product

MS in the CI mode confirmed that the GC product of metoprolol had a molecular weight of 279, 12 dalton higher than that of the parent compound. This suggests an incorporation of a methylene group into the molecule of metoprolol followed by a loss of two hydrogen atoms. Three structures are possible to account for this increase in molecular weight (IV), (V) and (VI) (Scheme 1).

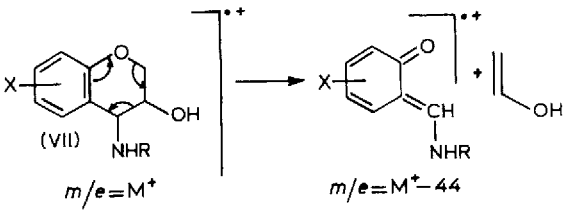
Although metoprolol and its GC product could be separated on a capillary column, the quantity of the compound formed was too small to be collected for structural elucidation. Facilities for the synthesis of postulated compounds (IV), (V) and (VI) in Scheme 1 were not available and an attempt was made to approach the structure of the product by means of GC–MS using isotopic properties of some deuterated solvents as isotopic "markers".

In the formation of the GC product, the hydroxyl and amino groups are the only reactive centres in the molecule of an aryloxypropanolamine (I), capable of undergoing a methylation at elevated temperature to give either a methoxy compound (II), route A, or a tertiary amine (III), route B.

In the next step the methoxy compound (II) could cyclise by eliminating two hydrogen atoms, one from the benzene ring and one from the methylene group next to the amino group, to form a dihydropyran ring. The resulting product would be a 4-isopropylamino-3-methoxychroman derivative (IV) (pathway a). The methoxy compound could also lose two hydrogen atoms, one from each of the methoxyl and amino groups of (II) to form an oxazolidine (V) (pathway b).

The tertiary amine (III) could cyclise in a similar way to give the same oxazolidine (V) (pathway c) and a 4-(N-isopropyl-N-methyl)aminochroman-3-ol derivative (VI) could be formed by a process similar to that involved in the formation of the chroman derivative (IV) (pathway d).

The mass spectral fragmentation of the chroman ring has been reported[15]. Mass spectral analysis of a series of *cis*- and *trans*-4-aminochroman-3-ols (VII) synthesised[16] as cyclic analogues of *β*-adrenergic blocking aryloxypropanolamines (I) revealed that the chroman derivatives (VII) fragmented by the loss of $CH_2=CH\text{-}OH$ according to the usual retro Diels–Alder fragmentation pathway (Scheme 2)[17].



Scheme 2.

The EI mass spectrum of the GC product under investigation (Fig. 2a) showed no such fragmentation and the structures (IV) and (VI) cannot be assigned to the compound of interest, leaving the oxazolidine derivative (V) as the most likely structure. The use of $C^2H_3O^2H$ as solvent shows that in the normal reaction (without deuterated reagents) a $-CH_2-$ group, but not a CH_3 group, is incorporated into the parent molecule as the molecular weight of the GC product increases from 279 to

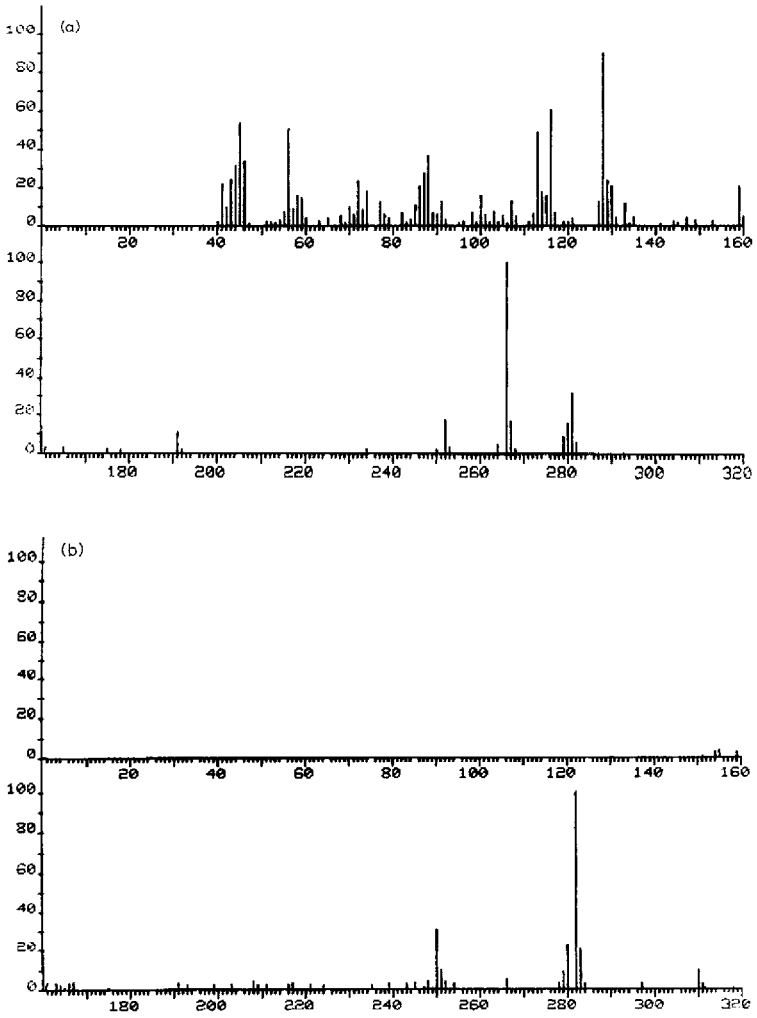


Fig. 3. (a) Metoprolol in $C^2H_3O^2H$, EI mass spectrum of GC product. (b) Metoprolol in $C^2H_3O^2H$, CI mass spectrum of GC product.

281 (Figs. 2b and 3b). To exclude the possibility that this increase in molecular weight could result from an exchange reaction between the deuterium atoms of the $-O^2H$ group and two hydrogen atoms in the original molecule, dichloromethane and its deuterated analogue were used as solvents. With dichloromethane, the GC product was found to be identical to that obtained with methanol, suggesting the elimination of two protons from the –OH and –NH groups as hydrogen chloride. Similarly, with $C^2H_2Cl_2$ the GC product was found to be identical to that obtained with $C^2H_3O^2H$.

A closer examination of the EI spectra of the GC product obtained with methanol and deuterated methanol provides additional evidence of the formation of the oxazolidine derivative. In the case of methanol, the ions *m/e* 114 and 86 correspond to the isopropyl oxazolidine and methyl oxazolidine fragments (Fig. 2). Similarly

with the deuterated methanol, the corresponding ions *m/e* 116 and 88 are present (Fig. 3a). The fragmentation pattern of the GC product agrees with that of a similar compounds formed by reacting metoprolol with phosgene[9].

Thus two pathways can apparently lead to the GC product (V). Pathway (A, b) proceeds via the formation of a methoxy compound (II) and pathway (B, c) via the formation of a tertiary amine (III). It is suggested that further investigation (such as synthesis of the postulated compounds II, III and V) is necessary to ascertain the exact pathway for the formation of the GC product. In the light of the above evidence, we suggest that the GC product of metoprolol in methanol is N-isopropyl-5-[4-(2-methoxyethyl)phenoxy]-methyloxazolidine (V).

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