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# The identification and analysis of mexiletine and its metabolic products in man

A. H. BECKETT* AND E. C. CHIDOMERE

*Pharmacy Department, Chelsea College, University of London, Manresa Road, London, SW3 6LX, U.K.*

Sensitive and specific gas-liquid chromatographic methods were developed for the analysis of mexiletine and its metabolites in urine of man. The identity of the g.l.c. peaks was established by mass-spectrometry. The hydroxylamine (Va) was qualitatively identified and determined quantitatively after conversion to the more stable oxime (Vb). Selective extraction procedures, t.l.c. and derivatization with hexamethyldisilazane (HMDS) and trifluoroacetic anhydride (TFA) were used in the qualitative identification of the major metabolites (VI–IX), particularly in distinguishing the basic products VI and VII from their corresponding alcoholic products VIII and IX. The limit of detection of the g.l.c. method was 6 to 12 ng ml$^{-1}$ for compounds I–IV, and 40 to 50 ng ml$^{-1}$ for compounds Vb–IX.

Mexiletine, Kö 1173, [1-(2′,6′-dimethyl)phenoxy-2-aminopropane] is a new antiarrhythmic drug (Allen, Ekue & others, 1972; Talbot, Nimmo & others, 1973; Campbell, Kelly & others, 1973; Campbell, Dolder & others, 1975). In previous studies, in rats and dogs, radioisotope techniques were used (Häselbarth & Pollmann, personal communication), while Kelly, Nimmo & others (1973) applied spectrophotofluorometric methods for the analysis of mexiletine in biological fluids. These methods are not sufficiently sensitive or specific for the determination of the range of metabolites produced in the presence of large amounts of unchanged drug. The g.l.c. method of Kelly & others (1973) and Kiddie & Kaye (1974) was used only for the determination of the unchanged drug. Hitherto only two major metabolites, the *p*-hydroxy (VI) and hydroxymethyl (VII) compounds have been identified (Häselbarth & Pollmann, personal communication).

In our studies, mexiletine was administered to healthy volunteers under various conditions of urinary pH and its metabolic products, excreted in urine, were identified, their properties investigated and methods devised for their analysis in mixtures in biological fluids.

## MATERIALS AND METHODS

### *Compounds and reagents*

Mexiletine (I) and its synthetic reference metabolites (compounds II–IX) were kindly synthesized by Boehringer Ingelheim Ltd. Benzophenone, benzyl ethyl ketone and furfurylamphetamine acetate were BDH chemicals; n-butylamphetamine hydrochloride was synthesized by Dr Haya of this department. *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, Pierce chemical Co.); hexamethyldisilazane (HMDS, Koch-Light Laboratories Ltd.); trifluoroacetic-anhydride (TFA, Aldrich Chemical Co. Inc.); acetonitrile (Fisons reagent), and β-glucuronidase-aryl sulphatase (Suc D'Helix Pomatia). Diethyl ether was freshly redistilled before use. All the compounds were checked for purity by g.l.c.

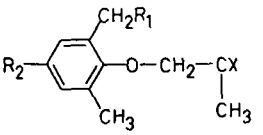


FIG. 1. Structures of metabolic products of mexiletine (I). (VI and VII are referred to in the text as *p*-hydroxymexiletine and hydroxymethylmexiletine respectively).

| | $R_1$ | $R_2$ | X | |
|---|---|---|---|---|
| I | H | H | $HNH_2$ | Mexiletine [1-(2′,6′-dimethyl)-phenoxy-2-aminopropane] |
| II | H | H | $HNHCH_3$ | *N*-methyl mexiletine |
| III | H | H | (= O) | 1-(2′,6′-Dimethyl)-phenoxypropan-2-one |
| IV | H | H | HOH | 1-(2′,6′-Dimethyl)-phenoxypropan-2-ol |
| Va | H | H | HNHOH | *N*-Hydroxymexiletine |
| Vb | H | H | (= NOH) | 1-(2′,6′-Dimethyl)phenoxy-propan-2-one oxime |
| VI | H | OH | $HNH_2$ | 1-(4′-Hydroxy,2′,6′-dimethyl)-phenoxy-2-aminopropane |
| VII | OH | H | $HNH_2$ | 1-(2′-Hydroxymethyl,6′-methyl)-phenoxy-2-aminopropane |
| VIII | H | OH | HOH | 1-(4′-Hydroxy,2′,6′-dimethyl)-phenoxypropan-2-ol |
| IX | OH | H | HOH | 1-(2′-Hydroxymethyl,6′-methyl)-phenoxypropan-2-ol |

### *Physical measurements*

Hydrogen-bonding studies of the synthetic oxime (Vb) were made by the dilution technique in spectrograde carbon tetrachloride, using a Perkin-Elmer

* Correspondence.

157G grating infrared spectrophotometer. Nuclear magnetic resonance (nmr) spectra of the oxime (Vb) were also recorded in $CDCl_3$ using a Perkin-Elmer R-10 nmr spectrometer, equipped with a Northern Scientific 544 CAT with tetramethylsilane as the internal standard.

All g.l.c.-mass spectra were obtained at an ionizing potential of 70 eV. Direct inlet spectra of reference compounds and isolated major metabolites were also obtained on a VG 12F Micromass spectrometer or an A.E.I. MS-9 mass spectrometer.

The g.l.c.-m.s. columns were: A, 1 m, 0·64 cm o.d. glass, with OV-17 (3%) on Chromosorb W, 80–100 mesh; temperature 150°–160°; helium (carrier gas), 60 $cm^3$ $min^{-1}$. B, 1 m, 0·64 cm o.d. glass, with Carbowax 20M (7·5%) on Chromosorb W, 80–100 mesh; temp. 175°–190°; helium 100 $cm^3$ $min^{-1}$.

*Gas-liquid chromatography*

A Perkin-Elmer F11 gas chromatograph equipped with a flame ionization detector and linked to a Hitachi Perkin-Elmer, 0–2·5 mV model 159 Recorder was used.

The g.l.c. systems were: A, 2 m, 0·64 cm o.d. glass, with OV-17 (3%) on Gas-Chrom Q, 80–100 mesh, acid-washed and dimethyldichloro silane-treated (AW-DMCS). The operating conditions were: $H_2$, 145 $kNm^{-2}$; air, 165 $kNm^{-2}$; $N_2$ (carrier gas), 69 $kNm^{-2}$ (60 $cm^3$ $min^{-1}$); column temperature 160°. B, 1 m, 0·64 cm o.d. glass, with Carbowax 20M (7·5%) on Chromosorb W, 80–100 mesh, AW-DMCS-treated. It was operated at two different column temperatures, i.e. $B_1$, 175°–185° and $B_2$, 125°–135°. At both temperatures, $H_2$, 172 $kNm^{-2}$; air, 150 $kNm^{-2}$, and $N_2$ (carrier gas), 103 $kNm^{-2}$ (100 $cm^3$ $min^{-1}$) were used. These columns were conditioned at 200° for 24 h and were silanized with 2 × 5 μl of hexamethyldisilazane (HMDS) before use.

*Thin-layer chromatography*

Glass plates (20 × 20 cm) were spread to a thickness of 0·25 mm with a mixture of silica gel G (Merck) and water (1:2). The plates were pre-dried at room temperature (30 min) and then oven-dried at 110° for 1 h before use. The solvent system used was benzene–methanol (80:20).

The following spray reagents were used to locate the various spots: Dragendorff's (I, II, VII); ammoniacal $AgNO_3$ (Tollen's, Va); aqueous cupric chloride, 1% (Vb); ferric chloride solution, 5% in 0·5N HCl (VI, VIII); Vanillin, 0·5% in 97% conc. $H_2SO_4$–methanol (80:20)–(IV, VI, VII, VIII, IX).

*Administration of drug to volunteers*

Mexiletine (I) was administered to four healthy males orally (200 mg base) and intravenously (100 mg base) under acidic (pH 4·8–5·0 see Beckett & Chidomere, 1977) and normal conditions of urinary pH. Urine samples were analysed qualitatively and quantitatively for metabolites.

*Instability of N-hydroxymexiletine (Va) in alkali*

Solutions of *N*-hydroxymexiletine (Va, 0·5 μmol $ml^{-1}$) were freshly prepared in distilled water or freshly collected blank human urine. 4 ml samples of each solution were distributed into eight separate centrifuge tubes. 1 ml 4 N NaOH was added and duplicate tubes of the aqueous and urine solutions were shaken for 30, 60, 90 and 120 min; the internal standard, benzophenone (9 μg $ml^{-1}$, 1 ml) was added and the solutions were extracted with freshly redistilled ether (3 × 3 ml). The concentrated extracts were subjected to both g.l.c. (system $B_1$) and t.l.c. analyses.

*Extractability of the phenolic compounds (VI and VIII) at different pH values*

A solution containing 50 μg $ml^{-1}$ each of *p*-hydroxymexiletine (VI) and its corresponding alcohol (VIII) was prepared using phosphate buffer, and 4 ml portions were distributed into eight centrifuge tubes and their pH adjusted to 4, 7, 8, 9, 10, 11, 12 and 12·5 respectively. Each solution was then extracted with freshly redistilled ether (3 × 3 ml); furfuryl-amphetamine solution in ether (11 μg $ml^{-1}$, 1 ml) was added as an external standard to the extracts. The ethereal extracts were concentrated to about 10–15 μl and their trifluoroacetic anhydride derivatives were prepared (5 μl TFA × 4 min) and analysed by g.l.c. system A.

*Quantitative analyses: extraction procedures*

*Analysis for unchanged mexiletine (I) in urine.* 4 ml of urine samples, collected at consecutive intervals after dosing, or blank urine to which the drug had been added, were placed in glass centrifuge tubes and 1 ml aqueous solution of n-butylamphetamine hydrochloride (10 μg $ml^{-1}$ of base) was added as an internal standard. After making alkaline (pH 12·9) with 1 ml 4N NaOH, the samples were extracted with freshly re-distilled ether (3 × 3 ml), shaking the tubes for 30 min on a mechanical shaker during each extraction. The combined ethereal extracts of each sample, in evaporating glass tubes with tapered bases, were concentrated (water bath at 43°) to about 20 μl. The concentrates (2–5 μl) were analysed by g.l.c. system $B_2$.

*Analysis for the free products of deamination (III) and keto-reduction (IV).* To 4 ml of the urine samples in centrifuge tubes was added 1 ml aqueous solution of benzyl ethyl ketone (0·1 $\mu$mol ml$^{-1}$), as internal standard. The pH was adjusted to 7–7·4 with dilute NaOH or HCl and 3 × 3 ml ethereal extractions were made as described for I. The concentrated extracts were analysed on g.l.c. system $B_2$.

*Analysis for hydroxymethylmexiletine (VII) and the total N-oxygenated products (Va and Vb) as the oxime (Vb).* Urine samples (4 ml) were made alkaline (pH 12·9) with 1 ml 4N NaOH and then shaken for 45 min. Benzophenone aqueous solution (9 $\mu$g ml$^{-1}$, 1 ml) was added as an internal standard and the samples were finally extracted three times with freshly redistilled ether. The concentrated extracts were analysed by g.l.c. system $B_1$.

*Analysis for the conjugated products (VI, VIII and IX).* Urine samples (4 ml) were placed in centrifuge tubes and the pH adjusted to 4·5 with dilute acetic acid. The samples were transferred to 25 ml Erlenmeyer flasks and 0·1 ml of enzyme $\beta$-glucuronidase/aryl sulphatase (Suc D'-Helix Pomatia) was added to each. The open flasks were incubated with shaking for 24 h at 37°. The contents were transferred to centrifuge tubes, 1 ml of aqueous marker solution of furfuryl-amphetamine acetate (11 $\mu$g ml$^{-1}$ base) was added and the pH adjusted to 10 with dilute NaOH or aqeuous $Na_2CO_3$. The samples were finally extracted with ether (3 × 3 ml) as for I, and the concentrated extracts were analysed on g.l.c. system A.

*Investigation for mexiletine excreted in the conjugated form.* After the extraction of the unchanged drug, the residual urine samples were combined and the volume measured. 6 or 10 ml aliquots were placed in 2 groups of 4–5 incubation flasks and the pH of their contents was adjusted to 4·5 with conc. HCl and dilute acetic acid. 0·1 ml of enzyme $\beta$-glucuronidase/aryl sulphatase was added to only one group of flasks and both groups were then incubated at 37° for 24 h. The contents were transferred to centrifuge tubes, 1 ml of n-butylamphetamine, 10 $\mu$g ml$^{-1}$ (internal standard) was added and the samples were extracted with ether (3 × 3 ml) after making alkaline with 1 ml 4 N NaOH. The concentrated ethereal extracts were analysed on g.l.c. column $B_2$.

*Calibration curves.* Standard solutions, of mixtures of the reference compounds (I–IX) were prepared in blank urine samples or in distilled water, the concentrations used were those found in urine after oral administration of the drug (I, 5–200 $\mu$g ml$^{-1}$; II, 0·1–1·0 $\mu$g ml$^{-1}$; III, IV and Vb, 1–10 $\mu$g ml$^{-1}$; VI–IX, 2·5–50 $\mu$g ml$^{-1}$ of base). Using the analytical procedures described above, calibration curves, based on the peak height ratios of the compounds to their internal standards, were obtained from 12 points representing 6 different concentrations in duplicate analyses. The data were subjected to computer (Olivetti T300) linear regression analysis to obtain the relevant calibration factors, slopes and correlation coefficients.

*Specificity of g.l.c. peaks.* All the analytical procedures described above had appropriate control

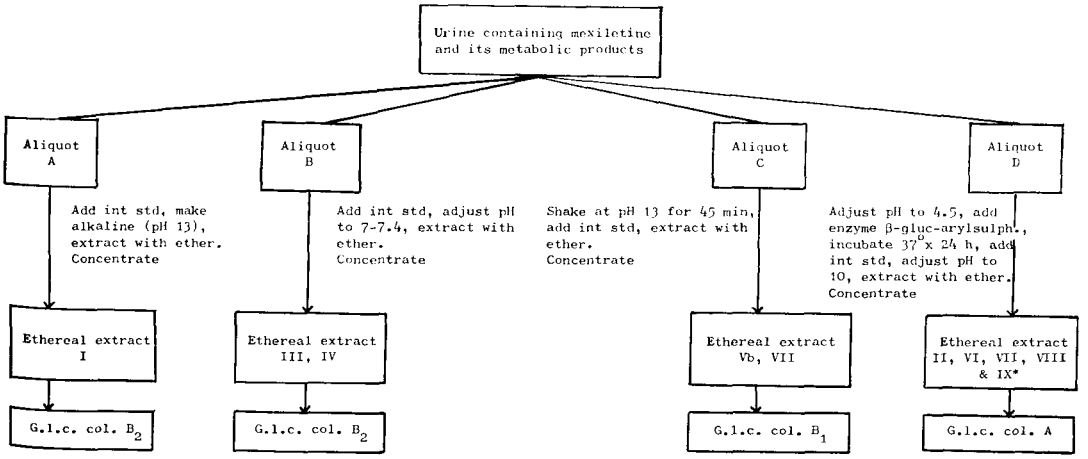


FIG. 2. Analytical scheme for the quantitative determination of mexiletine and its metabolic products in human urine. * IX was determined on this column only when VII was absent.

analyses of blank urine samples which had been collected from the subjects, used in the study, before the administration of mexiletine.

## RESULTS AND DISCUSSION

### *Identification of 2 new major metabolites (VIII and IX)*

Two new major metabolites of mexiletine, 1-(4′-hydroxy,2′,6′-dimethyl)phenoxy-propan-2-ol (VIII) and 1-(2′-hydroxymethyl, 6′-methyl) phenoxy-propan-2-ol (IX) were identified; their g.l.c., t.l.c. and mass-spectral characteristics were identical to those of the synthetic compounds. Both compounds were unaffected by treatment with $LiAlH_4$ and $TiCl_3$ reagents; both were extractable from acidic solutions, but only IX could be extracted at pH 12·5.

### *Extractability and stability of metabolites of mexiletine*

All the compounds (I–IX) could be extracted with diethyl ether from aqueous solutions. *N*-Hydroxymexiletine (Va) was unstable under the analytical conditions used, decomposing mainly to the oxime (Vb) but giving some parent amine (I), during g.l.c. analysis. Primary aralkyl-hydroxylamines are known to be relatively unstable during g.l.c. analysis (Beckett & Al-Sarraj, 1973; Beckett, Coutts & Ogunbona, 1973). The hydroxylamine (Va) was extracted at neutral pH (7–7·4). At alkaline pH (12·5) and with shaking, Va was converted to the oxime (Vb), as shown by t.l.c. G.l.c. analysis gave a peak height ratio of oxime (Vb) produced from the hydroxylamine (Va) identical to that obtained from the same concentration of synthetic oxime, thus representing a quantitative conversion (100 ± 1%) of Va to Vb. This treatment formed the basis for the quantitative determination of the *N*-oxidized metabolites.

The phenolic metabolites (VI and VIII) could be extracted at pH 9·5–10 (but not >12), where VI showed the optimum extractability.

### *Chromatographic characteristics (see Table 1)*

System B, used at different temperatures, was suitable for the analysis of most of the compounds, i.e. I–Vb and VII. However, system A was needed for the more polar compounds (VI, VIII and IX). Derivatizations with HMDS in dry acetonitrile and with TFA enhanced the separation of the major metabolites VI–IX; for while silylation did not separate the basic metabolites VI and VII from their corresponding alcoholic products VIII and IX, TFA derivatization separated the former from the latter, although the alcohols (VIII, IX) were not themselves separated (Table 1).

Table 1. *G.l.c. and t.l.c. characteristics of mexiletine, its metabolites and their derivatives.*

| | G.l.c. Rt (min) | | | | | |
|---|---|---|---|---|---|---|
| | Column A | | | Column | | |
| Comp. | Normal | TFA | HMDS | $B_1$ | $B_2$ | T.l.c. $R_F$ values |
| I | 3·5 | 6·8 | — | 2·0 | 10·0 | 0·35 (mid. p.) |
| II | 4·0 | 12·6 | — | 1·8 | 8·0 | 0·25 (mid. p.) |
| III | 3·5 | — | — | 2·2 | 12·0 | 0·73–0·75 |
| IV | 3·5 | 2·2 | | 3·0 | 18·0 | 0·63–0·65 |
| Va | 9·0 | D | 7·4 | 11·8 | — | 0·55 |
| Vb | 9·0 | D | 7·2 | *anti*-17·0 | — | 0·6–0·65 |
| | | | | *syn*-15·0 | | |
| VI | 18·0 | 11·4 | 14·0 | 100 | — | 0·12–0·15 (mid. p.) |
| VII | 14·0 | 10·0 | 12·0 | 21·0 | — | 0·12–0·15 (mid. p.) |
| VIII | 16·0 | 3·6 | 14·0 | | — | 0·44 (0·5) |
| IX | 14·0 | 3·6 | 12·0 | 34·0 | — | 0·74–0·87 (diffuse) |

D = decomposition.
G.l.c. derivatives: TFA, trifluoroacetic anhydride, HMDS, hexamethyldisilazane.
T.l.c. midpoint (mid. p.) $R_F$ values are quoted for compounds running as elongated spots. The locating reagents were: Dragendorff's (for I, II and VII, reddish-brown spots); Tollen's reagent (for Va, jet-black spot); cupric chloride (for Vb, yellowish-green, with heating); Vanillin (for IV, VI, brick-red; VII, bluish-green, with heating), and $FeCl_3$ (specific for VI and VIII, purple spots, with heating).

On t.l.c. the basic metabolites (VI, VII) had nearly identical $R_F$ values (0·15–0·2) near the base line; however the isolation and selective extraction procedures adopted (Fig. 3) made it possible to visualize their spots separately with minimum interference from each other.

Both the synthetic and metabolically produced oxime (Vb) under the analytical conditions of System $B_1$ (temperature 175°–185°) gave two peaks (Rt 15 and 17 min) for the geometrical isomers, the peak at the shorter retention time being 1/5–1/6 that at the longer retention time. A thermolytic rearrangement of the oxime to its *syn*-isomer had occurred at higher temperatures, as indicated by the following: (a) silylation (TMS) of the oxime gave a single symmetrical peak on the g.l.c.; (b) nmr spectra of the synthetic compound showed no isomeric peaks for the absorption due to methylene or methyl protons; (c) infrared studies of dilutions in $CCl_4$ showed a shift from the bonded –OH broad absorption at 3300 $cm^{-1}$ to the sharp free-OH absorption at 3600 $cm^{-1}$, thus indicating an intermolecular hydrogen bond consistent with an *'anti'*-oxime structure, for the oxime sample used; (d) the oxime mass spectra also showed that its hydroxyl hydrogen was at least in a sterically favoured position for it to be readily abstracted to form a prominent rearrangement ion (Beckett & Chidomere, unpublished).

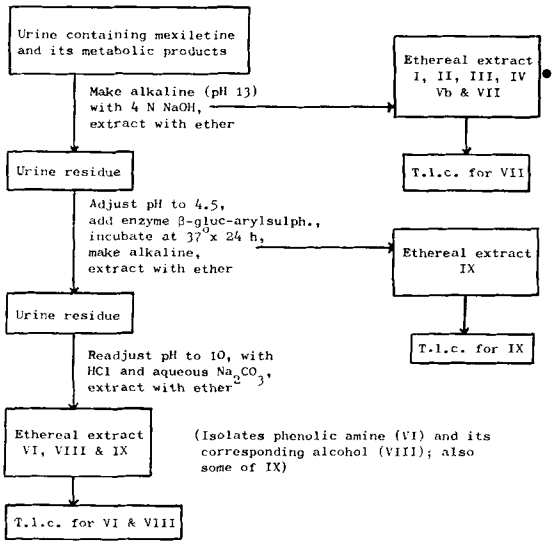


FIG. 3. Isolation and extraction from urine of the conjugated major metabolites (VI–IX) of mexiletine. * Remove mexiletine and products in their free forms.

*Mass spectral characteristics*

The mass spectra of both synthetic and metabolically produced compounds (I–IX) were identical.

Table 2. *Recoveries of mexiletine and its metabolites added to water (W) and urine (U).*

| Comp. | Concn ($\mu g\ ml^{-1}$) | | Recovery % (with s.d.) | |
|---|---|---|---|---|
| I | (U, W) | 5–200 | Mean* | 97·1 (3·0) |
| II | (U, W) | 0·1–1·0 | | 98–100 |
| III | (U, W) | 1·0–10 | | 98–100 |
| IV | (U, W) | 1·0–10 | | 98–100 |
| Vb | (W) | 10 | | 100·8 (4·9) |
| | | 8 | | 102·0 (1·4) |
| | | 2 | | 99·1 (6·3) |
| | (U) | 2–10 | | 100 (4·4) |
| VI | | 25 | W, 90·0 (6·0) | |
| | | 20 | W, 94·0 (4·8) | U, 94·0 (6·0) |
| | | 5 | W, 93·6 (5·8) | U, 90·0 (5·8) |
| VII | | 40 | W, 87·5 (8·5) | |
| | | 32 | W, 90·0 (7·8) | U, 90·0 (5·0) |
| | | 8 | W, 89·2 (4·8) | U, 88·0 (4·0) |

* Each result is the mean of 4 determinations for each concentration. Compounds II, III and IV were recovered virtually 100%, in the concentrations investigated, from both water and urine.

*Quantitative analysis*

Straight line calibration graphs (correlation coefficients 0·97–0·999) were obtained for all the compounds (I–IX) in both water and blank urine samples, using concentrations similar to those in which they were found in urine (see experimental section). Reproducible quantitative recoveries of the compounds were also obtained (Table 2) from their freshly prepared solutions in water and in urine from man.

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